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ORGANIC CHEMISTRY

ORGANIC CHEMISTRY

An Advanced Treatise

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VOLUME II

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PREFACE

Organic chemistry is richly endowed with excellent textbooks. However, there is a need for a general treatise of organic chemistry suitable for instruction at the graduate level. Such a book must focus attention upon new developments. At best, it can but serve the purpose of the moment and provide a point of departure for unceasing revision.

The idea of a collaborative work by specialists in the several branches of the science was developed in 1934. Each author was asked to prepare a chapter dealing with a subject of particular interest to himself. It was hoped to obtain, in this way, an authoritative treatise which would cover most of the important phases of organic chemistry. The execution of this plan has resulted in the present volumes.

For the sake of convenience in revising and expanding the book, the rapidly developing fields of natural products, relationship between physical properties and chemical constitution, valence, and resonance have been grouped together in the second volume. It is planned to revise both volumes at intervals, not only in order to bring the present material up to date, but also to permit the inclusion of new chapters to fill the more conspicuous gaps. For example, chapters on polymerization and chlorophyll will be included in the next edition. Corrections and suggestions will be heartily welcomed.

The contents have been integrated and the accessibility of the information increased by cross references, by individual tables of contents for each chapter, and by a comprehensive subject index which is repeated in each of the two volumes. The inordinate wealth of the literature has made it necessary to restrict references, in general, to a relatively few selected original articles. Researches are cited, as a rule, by reference to the most recent publications; however, sufficient references to early work are given to provide an historical background. Occasional chapters, particularly those in the field of natural products, have abundant citations to original articles, and should be especially useful to research workers. In some chapters the literature has been reviewed up to September, 1937. There is, in addition, occasional mention of work hitherto unpublished. The section General References

at the end of each chapter includes mention of some of the more important review articles and books as a guide to collateral reading.

The editors gratefully acknowledge the assistance of many friends in the examination of the manuscripts. Valuable aid was provided by the late Dr. W. H. Carothers, who served on the Editorial Board. Special thanks are due to Drs. G. E. Hilbert, J. F. Nelson, P. T. Parker, A. M. Patterson, G. F. Wright, and Messrs. J. C. Bailie, R. L. Bebb, L. C. Cheney, E. J. Crane, W. Harber, A. L. Jacoby, and J. Swislowky.

H. G.

AMES, IOWA
December, 1937

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CHAPTER 10

NATURAL AMINO ACIDS

H. T. CLARKE

Columbia University

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AMINO ACIDS FROM PROTEINS

The term protein connotes an ill-defined group of complex nitrogenous organic substances which form an important part of animal and vegetable tissues. The separation and characterization of individual "simple" proteins depend mainly on solubility relations, in accordance with which they are classified as albumins, globulins, and so forth. The simple proteins all yield ammonia and mixtures of amino acids on hydrolysis by acids, alkalis, or enzymes. "Conjugated" proteins also exist; these yield, besides amino acids, other products such as purines (p. 951), pyrimidines (p. 950), porphyrins, carbohydrates (or their derivatives), lipoidal substances, and phosphoric acid. Invariably, however, the principal products of hydrolysis consist of amino acids.

The most convenient method of hydrolysis involves treatment with hot aqueous mineral acids. The action of hot alkalis, though it readily brings about the desired hydrolysis, is less satisfactory, for during the process a notable proportion of the amino acids, which preëxist in pure optically active form, become racemized. This objection applies in a far less degree to acid hydrolysis. The action of proteolytic enzymes, though offering the practical disadvantage of slow and often incomplete

action, induces neither racemization nor decomposition of the more sensitive amino acids.

In proteins the constituent amino acids are united by peptide linkages ($-\text{CO}-\text{NH}-$ or, with the prolines (pp. 898, 906), $-\text{CO}-\text{N}<$), which on hydrolysis are opened with liberation of carboxyl and amino or imino groups. To follow the progress of hydrolysis, three methods are available: (1) titration of carboxyl groups, (2) titration of amino groups, (3) estimation of primary amino groups by treatment with nitrous acid. In the first two, conditions are so selected that the titration end points are influenced only by the groups to be estimated; in the third, a specific reaction is involved. The principles underlying the various procedures will be discussed later. On completion of hydrolysis, the resulting amino acids may be separated into three broad classes, which depend upon the preponderatingly acidic, basic, or neutral character of their members.

The predominantly acidic group consists of the monoamino dicarboxylic acids. These may be separated from the others by taking advantage either of the sparing solubility of their calcium or barium salts in aqueous alcohol, or of their selective tendency to migrate toward the positive pole when subjected in solution at suitable pH levels to the influence of an electric current.¹

The members of the predominantly basic group, comprising the diamino monocarboxylic acids, are characterized by their precipitability with phosphotungstic acid and by their tendency to migrate towards the negative pole in neutral solution.² The essentially neutral monoamino monocarboxylic acids, which constitute the major portion of most protein hydrolysates, differ from the members of the other groups * by the fact that they can be extracted from neutral solution by butyl alcohol.³ The majority of the members of this group, though appreciably soluble in butyl alcohol saturated with water, are insoluble in the anhydrous alcohol. Two amino acids of protein origin (proline and hydroxyproline), however, are distinguished by their solubility in pure alcohols; these also differ from all others in being not primary, but cyclic secondary amines. The group of "natural" monoamino monocarboxylic acids also includes a few which may be separated by virtue of their low solubility in water.

The following list, arranged on the basis of the above practical classi-

* Histidine (p. 933), in which the imidazole group possesses extremely weakly basic properties, forms an exception.

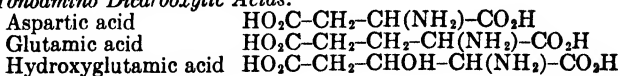
¹ Foster and Schmidt, *J. Biol. Chem.*, **56**, 545 (1923).

² Foster and Schmidt, *J. Am. Chem. Soc.*, **48**, 1709 (1926).

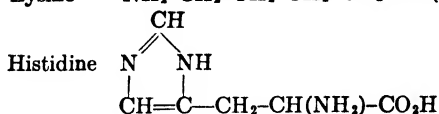
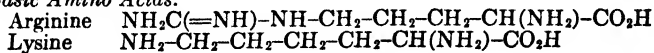
³ Dakin, *Biochem. J.*, **12**, 290 (1918); *J. Biol. Chem.*, **44**, 499 (1920).

fication, enumerates the amino acids which have been demonstrated to be products of the hydrolysis of proteins.⁴

I. *Monoamino Dicarboxylic Acids.*

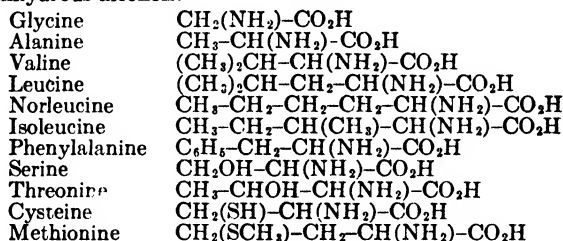


II. *Basic Amino Acids.*

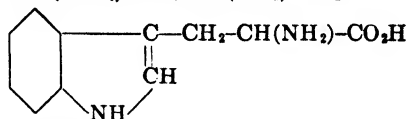


III. *Monoamino Monocarboxylic Acids.*

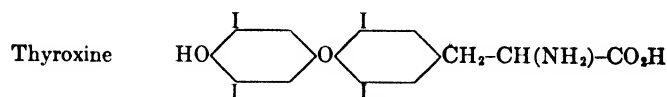
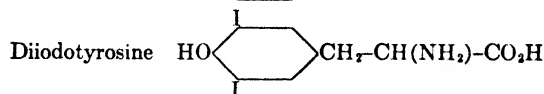
(1) Extractable by wet butyl alcohol; readily soluble in water; insoluble in anhydrous alcohols.



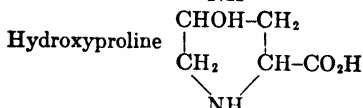
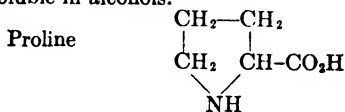
Tryptophan



(2) Sparingly soluble in water.



(3) Soluble in alcohols.



⁴ Vickery and Schmidt, *Chem. Rev.*, **9**, 169 (1931).

In addition to the above compounds of protein origin, certain other amino acids have been isolated from natural sources. Some of these will be discussed later.

The inclusion of cysteine in the above list is unconventional, as during the customary processes of isolation the sulfhydryl becomes oxidized to the disulfide, so that cysteine finally appears as cystine. The unquestionable presence of sulfhydryl groups in many proteins,⁵ however, points to the probability of the existence of cysteine as a component amino acid.

By acid hydrolysis, proteins yield considerable amounts of ammonia. There is reason to believe that this is derived from acid amide groups associated with the combined dicarboxylic acids, semi-amides of which have been isolated from the products of enzymatic hydrolysis of proteins.

Separation of the individual members of the first and second groups is effected by special methods involving selective precipitation of salts formed with metals or with acids. The quantitative aspects of these separations have been most completely developed for the basic amino acids (p. 921). The separation of the relatively simple monoamino monocarboxylic acids is rendered particularly difficult by the familiar similarity of members of homologous series, and it has not yet been found possible to develop quantitatively reliable methods for all. The original procedure of Fischer, fractional distillation of the ethyl esters under reduced pressure, involves notable losses due to formation of diketopiperazines.⁶ Attempts have been made to avoid this difficulty by acylation of the esters before fractionation,⁷ but these modifications await development to a state of practical utility.

Partial separation of the amino acids of a protein hydrolysate can be effected by taking advantage of the differential solubilities of their copper salts.⁸ Three fractions are obtainable: (1) soluble both in water and in methyl alcohol; (2) soluble in water, insoluble in methyl alcohol; (3) insoluble in either water or methyl alcohol. The amino acids obtained from a hydrolysate of gliadin were distributed as follows: (1) proline, hydroxyproline, valine, isoleucine; (2) glycine, alanine, serine, glutamic acid, hydroxyglutamic acid, the basic amino acids; (3) leucine, phenylalanine, tyrosine, aspartic acid.

The character, both physical and chemical, of proteins and peptides is largely determined by the nature and relative abundance of the various types of constituent amino acids. The polypeptides synthesized by

⁵ Mirsky and Anson, *J. Gen. Physiol.*, **18**, 307 (1935).

⁶ Fischer, *Ber.*, **34**, 433 (1901); *Z. physiol. Chem.*, **33**, 151 (1901); Foreman, *Biochem. J.*, **13**, 378 (1919).

⁷ Cherbuliez and collaborators, *Helv. Chim. Acta*, **12**, 317 (1929); **13**, 1290 (1930).

⁸ Town, *Biochem. J.*, **22**, 1083 (1928); Brazier, *ibid.*, **24**, 1188 (1930).

Fischer contained only monoamino monocarboxylic acids, and the only free acidic and basic groups present were those terminating the peptide chain. Proteins and natural polypeptides contain polar groups, situated at the uncombined ends of the acid and basic amino acids distributed throughout the molecule; the properties of the proteins represent a resultant of the individual and mutual effects of these.

GENERAL PROPERTIES AND REACTIONS OF NATURAL AMINO ACIDS

With the exception of proline and hydroxyproline, all the amino acids isolated from protein hydrolysates contain a primary amino group in the α -position to the carboxyl. The exceptions may be regarded also as α -amino acids in which the amino group is involved in ring formation; however, as may be judged from the solubility of proline in alcohol, this departure from the common form has a marked effect on physical as well as chemical properties.

With the exception of glycine, which contains no center of asymmetry, all the amino acids of protein origin occur in optically active form (p. 221). Evidence is accumulating that all possess the same spatial configuration. From approximately quantitative regularities in the molecular rotatory powers of corresponding derivatives of lactic acid and alanine, Freudenberg and his collaborators have concluded⁹ that natural alanine possesses the same configuration as *l*(+)-lactic acid. Analogous displacements of rotation are observed when groups (R) combined with the acid radical are varied in compounds containing the same substituents (R') on the amino and the hydroxyl group, respectively.



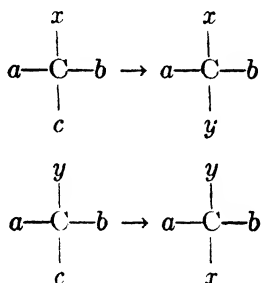
The rotatory powers of natural amino acids in neutral, acid, and alkaline solution often display marked differences which proceed in analogous directions with different members of the series; the values pass through a negative maximum at the isoelectric point and invariably become less levo- (or more dextro-) rotatory with increasing molar proportions of hydrochloric acid.¹⁰ This effect points to the probability of

⁹ Freudenberg and collaborators, *Ber.*, **57**, 1547 (1924); *Ann.*, **518**, 86 (1935).

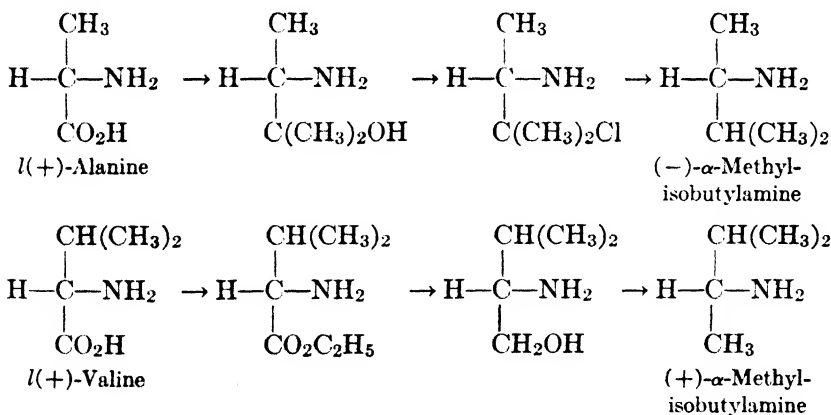
¹⁰ Wood, *J. Chem. Soc.*, **105**, 1988 (1914); Clough, *ibid.*, **107**, 1509 (1915); Levene and collaborators, *J. Biol. Chem.*, **81**, 687 (1929); Lutz and Jirgensons, *Ber.*, **63**, 448 (1930); **64**, 1221 (1931).

identical configuration, a conclusion supported by the observation of Karrer and Veer¹¹ that introduction of various acyl groups into natural leucine and valine and their esters causes parallel changes in optical rotation.

More direct evidence for the identity of the configurations of two "natural" amino acids has been secured by Barrow and Ferguson.¹² If two optically active compounds *Cabcr* and *Cabcy*, having the same configuration, each be converted into *Cabxy* by replacement of a common group *c* by *y* and *x*, respectively, the respective products, provided no Walden inversion has occurred, will possess opposite configurations.



This principle has been applied to the natural, dextrorotatory forms of alanine and valine.



The α -methylisobutylamine from the natural alanine formed a levorotatory hydrochloride; that from the valine was found to be dextrorotatory. During the syntheses some loss of activity occurred in each case, but since none of the atoms directly attached to the asymmetric carbon

¹¹ Karrer and Veer, *Helv. Chim. Acta*, **15**, 746 (1932).

¹² Barrow and Ferguson, *J. Chem. Soc.*, 410 (1935).

atoms was replaced during the processes, Walden inversions (p. 197, 1844) were not to be anticipated. Natural alanine and valine therefore possess the same (*l*) configuration.

The solubility relations of the simple α -amino monocarboxylic acids have been subjected to a critical study by Cohn and his collaborators.¹³ With increasing length of chain, the solubility in water decreases and the solubility in aqueous alcohol increases. In the homologous series, the difference between the logarithms of the solubility ratios for water and for absolute alcohol decreases by a constant amount for each additional methylene group. The substantial insolubility of amino acids, in general, in absolute alcohol and other organic liquids reflects the charged condition of the molecule. In alcohol-water systems containing small proportions of alcohol the logarithm of the molar solubility diminishes inversely as the dielectric constant. The effect of inorganic salts, and the mutual effect of different amino acids, present in the same solution, upon their individual solubilities are ascribable to their influence upon the dielectric constant of the solvent.

For every amino acid there is a definite value of *pH* at which it fails to migrate in solution to either pole when subjected to an electric current. This value, termed the isoelectric point, is that at which the molecule as a whole carries no unbalanced positive or negative charge. The isoelectric point coincides with the point of minimum solubility.

According to the classical theory, as employed, for example, in a study by Tague,¹⁴ this point, at which the net charge is effectively zero, was regarded as the *pH* level at which the dissociation of both the amino and the carboxyl group is at a minimum.

The modern theory¹⁵ takes the precisely opposite view. An aliphatic amino acid in solution at its isoelectric point is regarded as existing in its most highly charged condition with respect to its acidic and basic groups alike. This theory alone explains, for example, the effect of formaldehyde on the titration curves of amino acids. Addition of increasing amounts of formaldehyde to a solution of glycine causes a downward displacement of the curve in the region of higher *pH* but no change in that of lower *pH*; a similar effect is observed with ammonium acetate. Since, according to generally accepted views, the effect of the addition of alkali to ammonium salts is the suppression of basic ionization, it follows that in glycine, as in ammonium acetate, the upper portion of the titration curve relates to the basic function. In each case, therefore, the formaldehyde similarly suppresses the dissociation of the

¹³ Cohn, McMeekin, Edsall, and Weare, *J. Am. Chem. Soc.*, **56**, 2270 (1934).

¹⁴ Tague, *ibid.*, **42**, 173 (1920).

¹⁵ Bjerrum, *Z. physik. Chem.*, **104**, 147 (1923); Harris, *Biochem. J.*, **24**, 1080 (1930).

basic groups. With amino acids containing more than one amino group (e.g., lysine) the number of constituent curves characteristically shifted by addition of formaldehyde is equal to the number of basic groups present in the amino acid molecule; conversely, with monoamino dicarboxylic acids (e.g., aspartic acid) only one segment of the original titration curve is displaced, the two attributable to the carboxyl groups remaining unaltered. On the other hand, formaldehyde brings about little or no displacement in the upper (higher pH) portion of the titration curve of p -aminobenzoic acid, from which it is concluded that the aromatic amino group is only slightly dissociated.

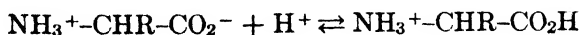
Aliphatic amino acids are therefore regarded as existing, in aqueous solution, largely in the form of molecules containing both positive and negative charges.



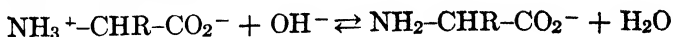
Such ions, the net charge of which is zero, have received the infelicitous name "Zwitterion" (from the German word *Zwitter*, meaning hermaphrodite). The expression "dipolar ion" is more acceptable to the linguistically sensitive than the hybrid term currently employed.

A solution of any given amino acid in pure water has not necessarily the pH corresponding to the isoelectric point of the amino acid; this would be the case only if the acid and basic functions had exactly the same tendency to assume the charged condition. In the simple α -amino acids, the carboxyl groups have a slightly greater tendency to part with their protons than the amino groups to accept them; as a result the hydrogen-ion concentration of their solutions is higher than that of water, but not sufficiently high to bring the total number of positive and negative charges on all the amino acid molecules into exact balance. This condition can be reached only by the addition to the solution of more hydrogen ions in the form of some acid. In the case of the monoamino monocarboxylic acids, the isoelectric points of which lie at approximately $pH = 6$, the discrepancy between the pH value of pure aqueous solutions and isoelectric point is but slight; it is much greater, of course, with the monoamino dicarboxylic acids. Conversely, the isoelectric point of the diamino monocarboxylic acids lies above $pH = 7$, and hydroxyl ions (in the form of alkali) must be added to their pure solutions to render them isoelectric.

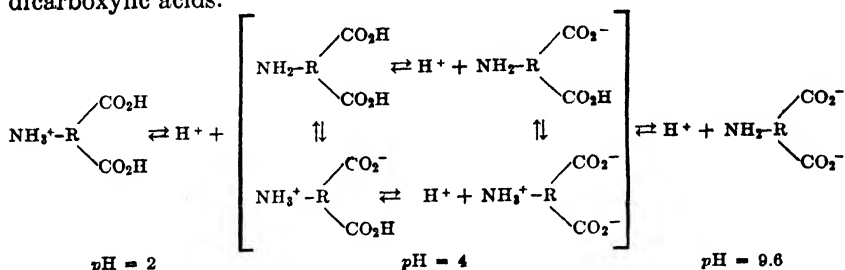
Addition of increasing amounts of mineral acid to a solution of an amino acid causes the suppression of the negative charge, until finally the equilibrium mixture contains the amino acid preponderantly in its purely cationic form.



Addition of alkali causes the suppression of the positive charge, with production of the anionic form.



The equilibria involved at different pH levels are illustrated by the case, discussed by Cohn in his admirable review,¹⁶ of the monoamino dicarboxylic acids.



That amino acids in their isoelectric range exist mainly in the dipolar ionic form is indicated by their Raman spectra.¹⁷ Fatty acids in aqueous solution (in which they are but weakly ionized) exhibit a line at about 1720 cm.⁻¹ characteristic of the carbonyl group; on the addition of sufficient alkali to cause almost complete ionization, this line vanishes. Amino acids fail to exhibit a line at this frequency, but do so when converted into their hydrochlorides. Conversely, free primary amines show strong Raman lines between 3300 and 3400 cm.⁻¹; lines in this region are not displayed by amino acids in their isoelectric zone, but appear on the addition of alkali.

Dipolar ions possess a large electric moment,¹⁸ particularly those of lysine and arginine, which exist in solution largely in the form of ions containing positive and negative charges at opposite ends of relatively long chains. Dicarboxylic amino acids, in isoelectric solution, exist mainly as less polar ions, resembling those of the simple α -amino acids, for their terminal carboxyl groups are less highly dissociated than those contiguous to the amino group. In solvents of low dielectric constant, such as 90 per cent alcohol, the concentration of highly polar ions is smaller, and that of uncharged molecules greater, than in water. For this reason it is possible, by the use of suitable indicators, to titrate independently either the acidic¹⁹ or the basic²⁰ function of amino acids in aqueous alcohol or acetone solution.

¹⁶ Cohn, *Ergeb. Physiol.*, **33**, 781 (1931).

¹⁷ Edsall, *J. Chem. Phys.*, **4**, 1 (1936); **5**, 225 (1937).

¹⁸ Edsall and Blanchard, *J. Am. Chem. Soc.*, **55**, 2337 (1933).

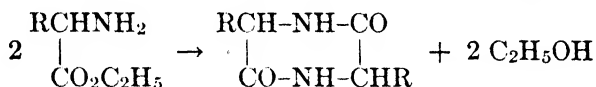
¹⁹ Foreman, *Biochem. J.*, **14**, 451 (1920); **22**, 208, 222 (1928).

²⁰ Linderstrøm-Lang, *Z. physiol. Chem.*, **173**, 32; **174**, 275 (1928).

The basic groups of amino acids can be quantitatively titrated in glacial acetic acid solution with perchloric acid in the same solvent.²¹ The titration may be carried out either potentiometrically by the method of Hall and Conant,²² or with the aid of a suitable indicator such as crystal violet.²³ The amino group behaves as a strong base, as in all aliphatic amines,²⁴ while the dissociation of the carboxyl group is completely suppressed by the solvent.

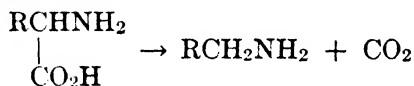
The dipolar character of the amino acids is reflected in their relative infusibility and low volatility. When strongly heated, they melt with profound decomposition²⁵ at temperatures well above 200°; some show a tendency to sublime below the decomposition point.²⁶

Since the negative character of the carboxyl group is suppressed by esterification, the amino acid esters are far more volatile than the amino acids. They display, nevertheless, a tendency to undergo condensation



with loss of alcohol. This tendency is more pronounced with the methyl and ethyl esters than with esters of higher alcohols such as butyl.²⁷ Analogous condensations undoubtedly take place, with loss of water, when amino acids are decomposed by heat.

Another type of decomposition which occurs on heating is decarboxylation.²⁸



This reaction takes place more readily in the presence of barium hydroxide or of a contact agent such as diphenylamine.²⁹ It also occurs when solutions of amino acids are exposed to the action of putrefactive organisms. Several of the amines so produced from natural amino acids are pharmacologically active; their formation in the lower intestine may be responsible for some forms of auto-intoxication.

²¹ Harris, *Biochem. J.*, **29**, 2820 (1935); *J. Biol. Chem.*, **84**, 296 (1929); Nadeau and Branchen, *J. Am. Chem. Soc.*, **57**, 1363 (1935).

²² Hall and Conant, *ibid.*, **49**, 3047 (1927).

²³ Conant and collaborators, *ibid.*, **49**, 3062 (1927); **52**, 4436 (1930).

²⁴ Hall and collaborators, *ibid.*, **50**, 2367 (1928); **52**, 5115 (1930).

²⁵ Dunn and Brophy, *J. Biol. Chem.*, **99**, 221 (1932).

²⁶ Brown, *Trans. Roy. Soc. Can.*, Sect. III, **26**, 173 (1932) [*C. A.*, **27**, 1617 (1933)].

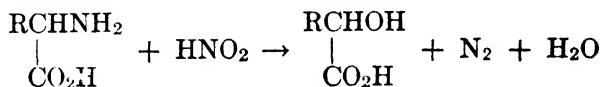
²⁷ Morgan, *J. Chem. Soc.*, 79 (1926).

²⁸ Cahours, *Ann.*, **109**, 10 (1859); Schulze and Barbieri, *Ber.*, **14**, 1785 (1881); **16**, 1711 (1883); Erlenmeyer and Lipp, *Ann.*, **219**, 161 (1883).

²⁹ Johnson and Daschavsky, *J. Biol. Chem.*, **62**, 725 (1925); Abderhalden and Gebelein, *Z. physiol. Chem.*, **152**, 125 (1926).

The usual functions of the carboxyl group in an amino acid are evident only under conditions in which the negative charge is suppressed. On treatment with alcohols, esterification takes place only in presence of an equivalent amount of a mineral acid, such as hydrogen chloride.³⁰ Amides are formed with great difficulty by heating amino acids with alcoholic ammonia;³¹ they are somewhat more readily produced by the action of alcoholic³² or anhydrous³³ ammonia upon amino acid esters. Chlorides of amino acids are capable of existing only in the form of salts, such as the hydrochloride. These have been prepared by treating a suspension of the amino acid in acetyl chloride with phosphorus pentachloride.³⁴ Esters of amino acids are reduced to the corresponding aldehydes by sodium amalgam.³⁵

α -Amino acids can, under suitable conditions, be made to undergo all the chemical reactions common to aliphatic primary amines. By the action of nitrous acid, for instance, they are converted into the corresponding hydroxy acids, with liberation of nitrogen.



This reaction forms the basis of the most specific and valuable analytical method³⁶ for the estimation of primary amino groups in amino acids, peptides, or proteins. It takes place quantitatively and rapidly by the action of acetic acid and sodium nitrite in excess; the nitric oxide which is simultaneously evolved by the reagents is removed by means of alkaline permanganate. Acid amide groups as a rule yield no nitrogen unless mineral acids are present, and ammonium salts buffered with sodium acetate react only very slowly. This reaction serves also to differentiate primary amines from the secondary variety (as in proline), from which no nitrogen is evolved. The guanidino group, which occurs in arginine, likewise yields no nitrogen unless mineral acid is present.

Acylation and similar processes are the most efficiently performed when the amino acid is in solution or in the form of a metallic salt. It seems probable that such reactions take place with amino groups only in their uncharged form, for it has been shown that in the solid state amino acids exist almost entirely in the form of electrically charged

³⁰ Curtius and Goebel, *J. prakt. Chem.*, [2] **37**, 150 (1888).

³¹ Heintz, *Ann.*, **150**, 67 (1869).

³² Franchimont and Friedmann, *Rec. trav. chim.*, **25**, 75 (1906).

³³ Koenigs and Mylo, *Ber.*, **41**, 4427 (1908).

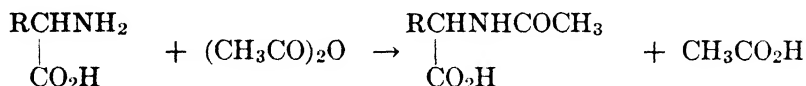
³⁴ Fischer, *Ber.*, **38**, 605, 2914 (1905).

³⁵ Neuberg, *Ber.*, **41**, 956 (1908); Fischer, *Ber.*, **41**, 1019 (1908).

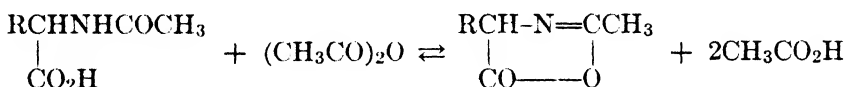
³⁶ Van Slyke, *J. Biol. Chem.*, **12**, 275 (1912).

dipoles.³⁷ In simple aqueous solution the proportion of uncharged amino groups in equilibrium with the positively charged ionic groups is sufficient to permit acylation reactions to proceed at a slow rate; in the presence of added alkali this proportion is greatly increased, and acylation is facilitated. The principle is illustrated by the need for alkaline conditions during the introduction of phenylureido, arylsulfonyl, or benzoyl groups into amino acids.³⁸

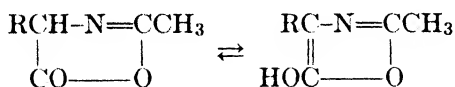
Acetylation of natural amino acids leads, under certain conditions, to loss of optical activity. When the reaction is carried out in cold acetic acid or in alkaline solutions by means of the theoretically necessary amount of acetic anhydride, optically active acetamino acids are produced.



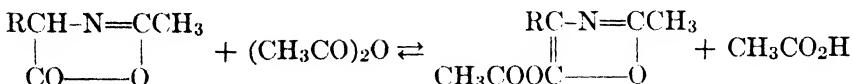
These are, however, racemized by heating in acetic acid solution with small quantities of acetic anhydride, while with large amounts they are converted into inactive azlactones. These observations³⁹ can be explained by postulating an equilibrium between acetamino acids and acetic anhydride,



and the enolization of the azlactone present in equilibrium.



This enolization, which appears to be catalyzed by acetic anhydride,



must take place relatively slowly, for when an optically active amino acid is treated with excess of acetic anhydride in presence of ammonium thiocyanate, an optically active acetyl thiohydantoin results.⁴⁰ The

³⁷ Cohn, *Ann. Rev. Biochem.*, **4**, 93 (1935).

³⁸ Baum, *Z. physiol. Chem.*, **9**, 465 (1885); Fischer, *Ber.*, **32**, 2451 (1899); Fischer and Bergell, *Ber.*, **35**, 3779 (1902).

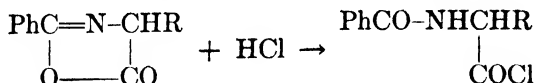
³⁹ Bergmann and Zervas, *Biochem. Z.*, **203**, 280 (1928).

⁴⁰ Csönka and Nicolet, *J. Biol. Chem.*, **99**, 213 (1932).

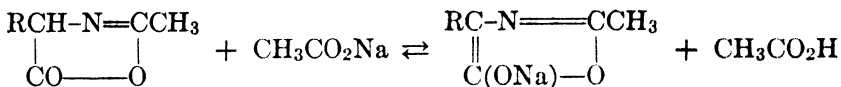
formation of this type of compound is due to a reaction, presumably more rapid than the enolization, involving an azlactone.⁴¹



The addition of thiocyanic acid to the azlactone finds an analogy in the reaction between azlactones and hydrogen chloride.⁴²

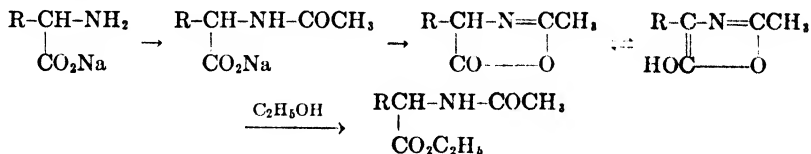


Although the acetyl group can be introduced without racemization by the action of acetic anhydride in excess upon amino acids dissolved in boiling water,⁴³ the same reaction carried out in presence of sodium acetate at 35–40° leads to a completely racemized acetamino acid.⁴⁴ This is explained as being due to the induction by the sodium acetate of enolization of the azlactone transitorily formed from the acetamino acid by the action of acetic anhydride.



Pyridine acetate acts in the same way, for sodium acetate and pyridine behave as strong bases in acetic acid solution, but sodium chloride is without effect. The presence of an alkyl group on the nitrogen effectively prevents racemization.

When the sodium salt of an amino acid is treated in alcohol with acetic anhydride,⁴⁵ an acetylated ester of a racemized amino acid is produced.



When proline is subjected to these conditions, acetylation alone occurs, accompanied by neither racemization nor esterification.

The effect of sodium acetate in promoting the enolization and conse-

⁴¹ Johnson and Scott, *J. Am. Chem. Soc.*, **35**, 1136 (1913).

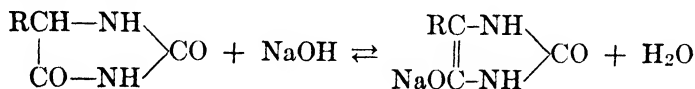
⁴² Mohr, *Ber.*, **42**, 2521 (1909); *J. prakt. Chem.*, **81**, 49, 473; **82**, 322 (1910).

⁴³ Behr and Clarke, *J. Am. Chem. Soc.*, **54**, 1630 (1932).

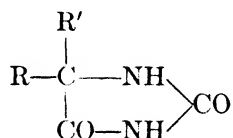
⁴⁴ du Vigneaud and Meyer, *J. Biol. Chem.*, **98**, 295; **99**, 143 (1932).

⁴⁵ Ashley and Harington, *Biochem. J.*, **23**, 1178 (1929).

quent racemization of azlactones dissolved in acetic acid, even though this solvent is diluted by water or alcohol, finds an analogy in the observation of Dakin⁴⁶ that hydantoins of natural amino acids undergo spontaneous racemization in presence of alkali at room temperature.

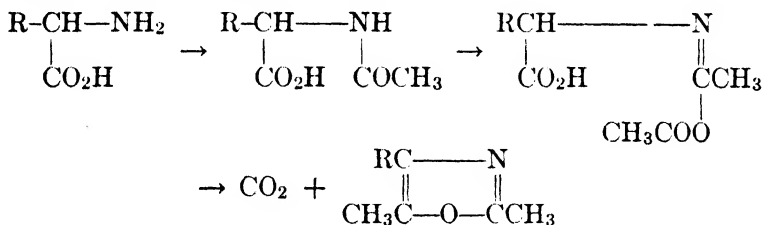


The racemization, which does not occur with the corresponding hydantoic acids nor with hydantoins of the type

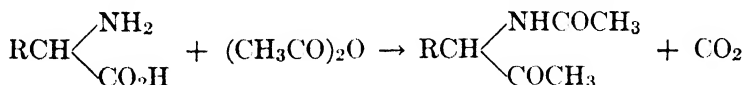


is ascribable to enolization.

When heated with acetyl chloride in acetic acid, α -amino acids are converted into derivatives of oxazole.⁴⁷



An equally profound, and probably related, reaction occurs when α -amino acids are warmed with acetic anhydride in presence of pyridine,⁴⁸ methyl acetaminoalkyl ketones being produced.



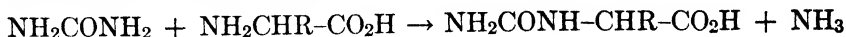
Here again it appears that an enolizable derivative is involved as an intermediate, for under the same conditions proline and N-alkylamino acids are merely acetylated, and no corresponding ketone is formed from α -amino- α -phenylpropionic acid.

⁴⁶ Dakin, *Am. Chem. J.*, **44**, 48 (1910).

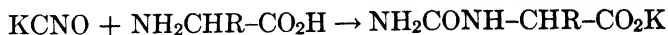
⁴⁷ Wrede and collaborators, *Z. physiol. Chem.*, **203**, 279 (1931); **206**, 146 (1932); **218**, 129 (1933).

⁴⁸ Levene and Steiger, *J. Biol. Chem.*, **74**, 689 (1927); **79**, 95 (1928); Dakin and West, *ibid.*, **78**, 91, 757 (1928).

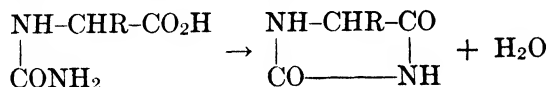
On treatment with a boiling solution of urea, α -amino acids yield the corresponding hydantoic acids.⁴⁹



During the process racemization takes place. This may be avoided by the use of potassium cyanate.⁴⁶

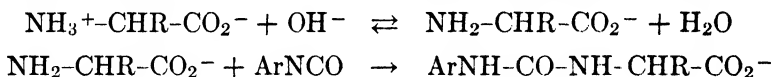


The resulting hydantoic acids readily undergo ring closure to the hydantoin on boiling with hydrochloric acid,



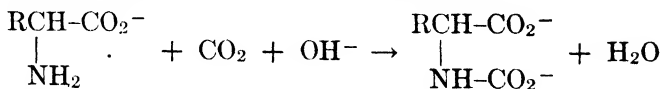
a reaction which would proceed in the reverse direction if the reacting groups could not come into steric propinquity.

Substituted hydantoic acids are formed by the action of aromatic isocyanates upon amino acids in aqueous solution. The reaction, which takes place in the cold, is favored by the presence of alkali;⁵⁰ this suppresses the dissociation of the basic group, thereby increasing the proportion of the anionic form with which the isocyanate reacts.



The introduction of acyl or ureido groups abolishes the amphoteric character of amino acids; the products are soluble in non-polar organic liquids, and are suitable derivatives for identification.

When carbon dioxide is passed into alkaline solutions of amino acids, salts of corresponding carbamino acids are produced.⁵¹



The calcium and barium salts are sparingly soluble in dilute alcohol; in boiling water they break down into the amino acids and metal carbonate. An attempt has been made⁵² to exploit the differences in solubility of the barium salts of the carbamino acids for the systematic separation of the products of protein hydrolysis.

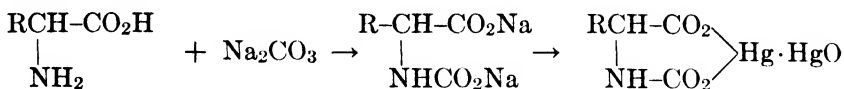
⁴⁹ Lippich, *Ber.*, **39**, 2953 (1906); **41**, 2976 (1908).

⁵⁰ Paal, *Ber.*, **27**, 974 (1894); Neuberg and Manasse, *Ber.*, **38**, 2359 (1905).

⁵¹ Siegfried and collaborators, *Z. physiol. Chem.*, **44**, 85 (1905); **46**, 401 (1905); **54**, 423 (1908); **65**, 295 (1910); **81**, 260 (1912); *Ber.*, **39**, 397 (1906); Stadie and O'Brien, *J. Biol. Chem.*, **112**, 723 (1936).

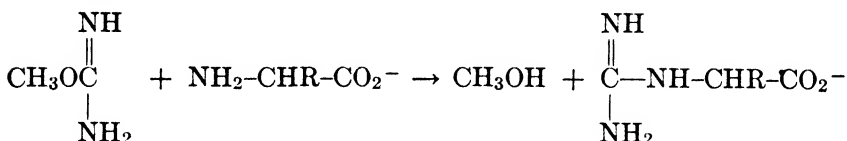
⁵² Schryver and collaborators, *Biochem. J.*, **15**, 636 (1921); **18**, 1070 (1924).

Basic mercury salts of carbamino acids are formed when mercuric acetate is added to solutions of amino acids made—and maintained—alkaline with sodium carbonate.⁵³



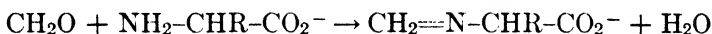
In most instances these salts are nearly quantitatively precipitated on the addition of alcohol.

Amino acids are converted into the corresponding guanidino acids by treatment with O-methylisourea,⁵⁴

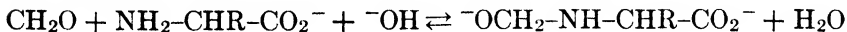


or S-methylisothiurea.

Reference has already been made (p. 866) to the use of formaldehyde in the titration of amino acids. Sørensen,⁵⁵ who developed the process as an analytical method, ascribed the suppression of the basic functions of the amino group to the establishment of an equilibrium involving methylene compounds of the type formulated by Schiff.



However, the reaction appears to be more complicated. Metallic salts of such condensation products have been prepared which contain the elements of one or more additional molecules of water or formaldehyde,⁵⁶ so that it seems probable that in solution an equilibrium exists between the methylene, methylol, dimethylol, and more complex forms. On the other hand, determination of the equilibrium constant of the reaction between amino acids and formaldehyde⁵⁷ points to the formation, over the range pH 8 to 10, of equimolar compounds only.



Of the few salts which have been prepared in crystalline condition, the barium compound from formaldehyde and glycine has proved⁵⁸ to

⁵³ Neuberg and Kerb, *Biochem. Z.*, **40**, 498 (1912).

⁵⁴ Kapfhammer and Müller, *Z. physiol. Chem.*, **225**, 1 (1934).

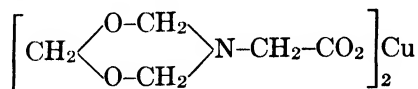
⁵⁵ Sørensen, *Biochem. Z.*, **7**, 45 (1907).

⁵⁶ Franzen and Fellmer, *J. prakt. Chem.*, [2] **95**, 299 (1917); Krause, *Ber.*, **51**, 136, 542, 1556 (1918); **52**, 1211 (1919).

⁵⁷ Tomiyama, *J. Biol. Chem.*, **111**, 51 (1935).

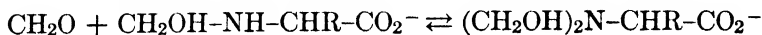
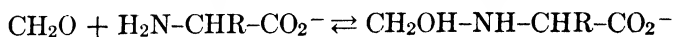
⁵⁸ Bergmann and Ensslin, *Z. physiol. Chem.*, **145**, 194 (1925).

possess the methylene structure $(\text{CH}_2=\text{N}-\text{CH}_2-\text{CO}_2)_2\text{Ba}$, while the crystalline copper salt has the structure:



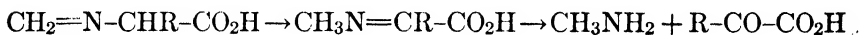
Well-defined compounds analogous to this last are formed from glycine ester with excess of formaldehyde, and from aliphatic amines and aldehydes in general.⁵⁹

Studies of titration curves⁶⁰ of amino acids with increasing concentrations of formaldehyde indicate the formation of dimethylol derivatives



and that only in the latter are the basic properties suppressed to the point at which they are no longer discernible in the titration. Proline, which is incapable of forming a Schiff base or a dimethylol derivative, exhibits appreciable basic dissociation in presence of even a large excess of formaldehyde.

When heated in acid solution with formaldehyde, amino acids [with the exception of glycine, which is converted into methylene diglycine, $\text{CH}_2(\text{NH}-\text{CH}_2-\text{CO}_2\text{H})_2$]⁶¹ undergo extensive decomposition, a large proportion of their nitrogen being liberated in the form of methylamine.⁶² It appears probable that this decomposition involves the transposition of the double bond of a Schiff base.⁶³



Similar decompositions are brought about by *o*-quinones,⁶⁴ methylglyoxal,⁶⁵ sugars,⁶⁶ isatin,⁶⁷ and α -keto acids.⁶⁸ In the last instance, the condensation product undergoes rearrangement with simultaneous decarboxylation, followed by hydrolysis. These reactions may proceed in two directions, with formation of the aldehyde derived either from

⁵⁹ Bergmann and collaborators, *ibid.*, **131**, 18 (1923); *Ber.*, **57**, 662 (1924).

⁶⁰ Levy, *J. Biol. Chem.*, **99**, 767 (1933).

⁶¹ Löb, *Biochem. Z.*, **51**, 116 (1913).

⁶² Zeleny and Gortner, *J. Biol. Chem.*, **90**, 427 (1931).

⁶³ Clarke, Gillespie and Weisshaus, *J. Am. Chem. Soc.*, **55**, 4571 (1933).

⁶⁴ Kisch and collaborators, *Biochem. Z.*, **242**, 1 (1931); **244**, 440; **247**, 371; **249**, 63 (1932).

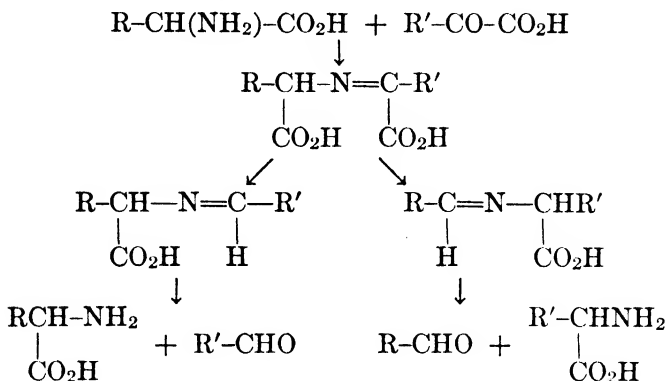
⁶⁵ Kisch, *ibid.*, **257**, 334 (1933).

⁶⁶ Akabori, *Ber.*, **66**, 143 (1933).

⁶⁷ Franke, *Biochem. Z.*, **258**, 296 (1933).

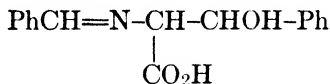
⁶⁸ Herbst and Engel, *J. Biol. Chem.*, **107**, 505 (1934); Herbst, *J. Am. Chem. Soc.*, **58**, 2239 (1936).

the amino acid or from the keto acid, or both. When the former aldehyde is produced, a new amino acid is also formed.

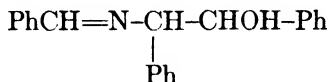


The nature of the substituents R and R' appears to determine which of the two carboxyl groups is eliminated.

Aromatic aldehydes yield condensation products with amino acids in presence of alkali⁶⁹ yielding Schiff bases $\text{Ar-CH=N-CHR-CO}_2\text{Na}$ in which the double bond, being conjugated with the aromatic nucleus, shows less tendency to migrate and thereby to initiate further decomposition of the kind observed with aliphatic aldehydes. Subsequent condensations may, however, take place with further quantities of the aromatic aldehyde;⁷⁰ glycine and benzaldehyde yield N-benzylidene phenyl serine,



together with a by-product in which the carbon structure of the amino acid does not reappear.



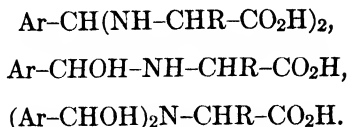
Measurements of optical activity⁷¹ indicate that under milder conditions, in cold aqueous alcohol at pH 9-10, reversible equilibrium

⁶⁹ Gerngross, *Biochem. Z.*, **108**, 89 (1920); Gerngross and Zublke, *Ber.*, **57**, 1482 (1924); Bergmann and collaborators, *Ber.*, **58**, 1034 (1925); *Z. physiol. Chem.*, **152**, 282 (1926); **172**, 277 (1927).

⁷⁰ Erlenmeyer and collaborators, *Ber.*, **25**, 3445 (1892); **28**, 1866 (1895); **30**, 1527, 2896 (1897); *Ann.*, **284**, 36 (1894); **307**, 79, 113 (1899); **337**, 205 (1904).

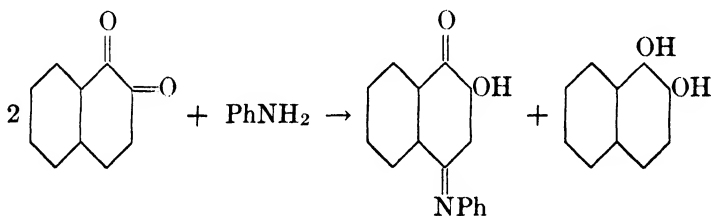
⁷¹ Gulland and Mead, *J. Chem. Soc.*, 210 (1935).

reactions take place between aromatic aldehydes and amino acids, with formation of compounds such as

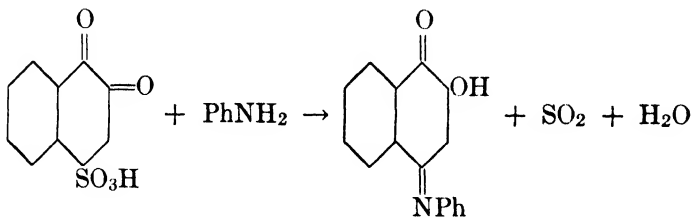


The maximum change of rotation is usually reached with 2-3 moles of the aldehyde.

The action of β -naphthoquinone and the closely related 1,2-naphthoquinone-4-sulfonic acid upon α -amino acids is of practical as well as theoretical interest. With aniline, both of these compounds are converted into 2-hydroxy-1-naphthoquinone-4-anil, a red substance which resists the reducing action of sulfurous acid. In the first case,⁷² part of the quinone is reduced to the hydroquinone;



in the second,⁷³ the sulfonic group is eliminated as sulfur dioxide.



Amino acids appear to act similarly, yielding red solutions, the color of which, in contrast to that of the quinone reagent, is not discharged by thiosulfate.⁷⁴ The intensity of the color developed with β -naphthoquinonesulfonic acid is, with almost all the natural amino acids, proportional to their molecular concentration, so that the test can be applied for the quantitative estimation of amino acids in general. With the

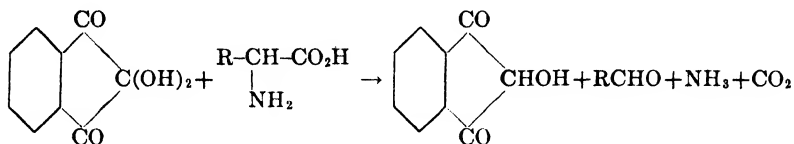
⁷² Liebermann, *Ber.*, **14**, 1310 (1881); Zincke, *Ber.*, **14**, 1493 (1881); Liebermann and Jacobson, *Ann.*, **211**, 36 (1882).

⁷³ Böniger, *Ber.*, **27**, 23 (1894).

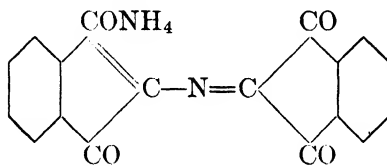
⁷⁴ Folin, *J. Biol. Chem.*, **51**, 377, 393 (1922).

exception of ammonia, none of the usual metabolic waste products interferes; ammonia can first be eliminated by means of permutite.

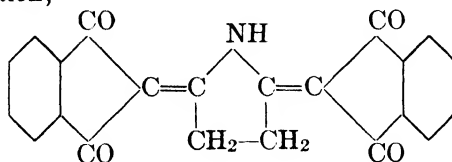
The "ninhydrin" reaction, a sensitive color test in which a blue color is developed on warming amino acids with triketohydrindene hydrate in dilute aqueous solution, involves oxidative deamination.⁷⁵ The first step consists in the dehydrogenation of the amino acid, which passes over into ammonia and an aldehyde.



Triketohydrindene and its reduction product then condense with ammonia to yield the blue coloring matter,



the constitution of which is analogous to that of murexide. Identical color intensities are developed with equimolar solutions of all α -amino acids and other compounds, such as dipeptides and aminoacetone, which contain α -aminoacyl groups.⁷⁶ Proline and hydroxyproline yield with triketohydrindene a different type of condensation product, in which only the carboxyl group has been eliminated.⁷⁷ That from proline has the constitution;



and possesses a red color.

Amino acids, like simple amines, are converted by sodium hypochlorite into N-chloro derivatives, the process being almost independent of concentration.⁷⁸ Esters of amino acids behave similarly. The

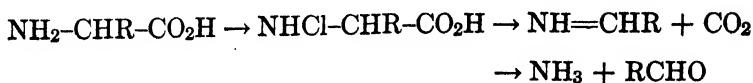
⁷⁵ Ruhemann, *J. Chem. Soc.*, **97**, 2025 (1910); **99**, 792, 1486 (1911); Abderhalden and Schmidt, *Z. physiol. Chem.*, **72**, 37 (1911); Harding and Warneford, *J. Biol. Chem.*, **25**, 319 (1916); Retinger, *J. Am. Chem. Soc.*, **39**, 1059 (1917).

⁷⁶ Cherbuliez and Herzenstein, *Helv. Chim. Acta*, **17**, 1440 (1934).

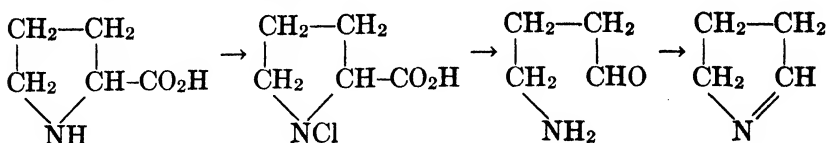
⁷⁷ Grassmann and v. Arnim, *Ann.*, **509**, 288 (1934).

⁷⁸ Langheld, *Ber.*, **42**, 2360 (1909).

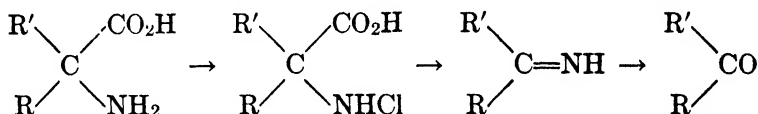
resulting products break down, slowly in the cold but rapidly on warming, into ammonia, carbon dioxide, and an aldehyde.



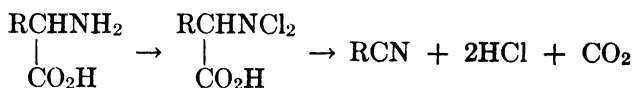
The reaction proceeds similarly with secondary amino acids; sarcosine yields methylamine in place of ammonia, proline breaks down into carbon dioxide and pyrrole.



The presence of two alkyl groups or of an acyl group on the nitrogen atom inhibits the action of hypochlorite. Amino acids completely substituted in the α -position (e.g., α -aminoisobutyric acid) are, on the other hand, readily oxidized to ketones.



Chloramine T (sodium *p*-toluenesulfonchloroamide) in neutral solution acts in the same way as hypochlorite,⁷⁹ yielding aldehydes, carbon dioxide, and ammonia when equimolecular quantities of the reactants are employed. With two moles of Chloramine T, on the other hand, a different reaction occurs⁸⁰ whereby nitriles are formed.



Both types of reaction occur when amino acids are oxidized with sodium hypobromite, the formation of aldehyde being favored, at the expense of nitrile production, by high alkalinity.⁸¹

Amino acids are oxidized by peroxides⁸² or persulfates⁸³ with formation of aldehydes. Oxygen in presence of charcoal, palladium

⁷⁹ Dakin and collaborators, *Proc. Roy. Soc. (London)*, **B89**, 232 (1916); *Biochem. J.*, **11**, 79 (1917).

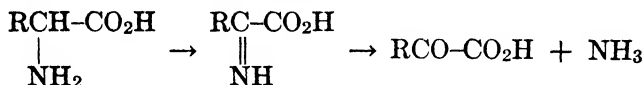
⁸⁰ Dakin, *Biochem. J.*, **10**, 319 (1916).

⁸¹ Friedman and Morgulis, *J. Am. Chem. Soc.*, **58**, 909 (1936).

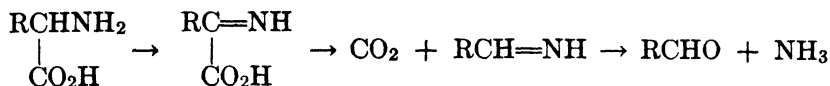
⁸² Dakin, *J. Biol. Chem.*, **1**, 171 (1905); **4**, 63 (1908); **5**, 409 (1909).

⁸³ Lang, *Z. physiol. Chem.*, **241**, 68 (1936).

black,⁸⁴ or finely divided iron⁸⁵ causes their breakdown to aldehydes or ketones. The oxidation of amino acids by biological systems proceeds through the α -keto acid stage,⁸⁶ and therefore probably involves dehydrogenation followed by hydrolysis,



a process which has been shown⁸⁷ to be biochemically reversible. As the formation of pyruvic acid by the action of oxygen upon alanine in presence of charcoal could not be detected, the breakdown was at first⁸⁴ formulated differently.



This view was supported by the failure of α -aminoisobutyric acid, which is structurally incapable of such dehydrogenation, to undergo oxidation under the same conditions. It was, however, subsequently shown⁸⁸ that α -dimethylaminoisobutyric acid is readily oxidized by oxygen in presence of charcoal, yielding acetone, carbon dioxide, and dimethylamine, so that the dehydrogenation theory for such oxidations had to be abandoned in favor of the idea that an addition compound with oxygen is formed. It was also found that ozone, which had previously been reported⁸⁹ to be without action on aliphatic amino acids, breaks down not only the natural amino acids, but dialkylamino acids, α -aminoisobutyric acid, and α -dimethylaminoisobutyric acid into aldehyde (or ketone), carbon dioxide, and volatile base. Similar products are formed from all the same types of compound by the action of silver oxide,⁹⁰ with which mono- and dimethylamino acids react more readily than the primary compounds; betaine and β -alanine are not attacked.

Amino acids are not oxidized by methylene blue alone, but are degraded to aldehydes by palladium black in the presence of a hydrogen acceptor such as alloxan or dinitrobenzene.⁸⁴ Charcoal is without action in the entire absence of oxygen.⁹¹

⁸⁴ Wieland and Bergel, *Ann.*, **439**, 196 (1924).

⁸⁵ Handovsky, *Z. physiol. Chem.*, **176**, 79 (1928).

⁸⁶ Neubauer and collaborators, *ibid.*, **67**, 230 (1910); **70**, 326 (1911).

⁸⁷ Knoop, *ibid.*, **67**, 489 (1910); Knoop and Kertess, *ibid.*, **71**, 252 (1911); Neber, *ibid.*, **234**, 83 (1935).

⁸⁸ Bergel and Bolz, *ibid.*, **220**, 20 (1933).

⁸⁹ Harries and Langheld, *ibid.*, **51**, 373 (1907).

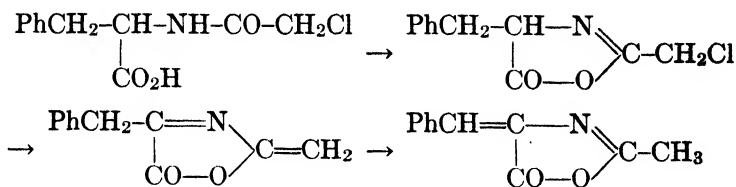
⁹⁰ Herbst and Clarke, *J. Biol. Chem.*, **104**, 769 (1934).

⁹¹ Wieland, Drishaus, and Koschura, *Ann.*, **513**, 203 (1934).

Amino acids are deaminated on exposure to ultra-violet light;⁹² the reaction is specific for the α -amino grouping⁹³ and takes place in neutral, acid, or alkaline solution. Little is known of its mechanism.

The oxidative deamination of amino acids by biological systems, an important vital process, has been elucidated by the work of Krebs.⁹⁴ When solutions of amino acids are exposed to oxygen in the presence of slices of organ tissue, ammonia and the corresponding α -keto acid are formed. Kidney tissue is the most active, liver somewhat less so. With the natural (*l*) amino acids no reaction occurs in the absence of oxygen, if the tissue be ground or dried, nor in the presence of octyl alcohol or cyanide. On the other hand, the amino acids of opposite (*d*) configuration are not only deaminated more rapidly by intact slices of tissue, but the reaction is inhibited neither by drying, grinding, nor the addition of the above poisons. It is therefore concluded that two distinct enzyme systems are respectively responsible for the oxidative deamination in the kidney and liver of amino acids belonging to the two configurational groups.

Dehydrogenation of amino acids to derivatives of the corresponding unsaturated amino acids has been effected by means of a remarkable reaction discovered by Bergmann and Stern.⁹⁵ When chloroacetyl-phenylalanine is warmed with acetic anhydride, dehydration occurs; the product, however, has lost the elements of hydrogen chloride and consists of α -acetaminocinnamic azlactone, identical with the product of the action of benzaldehyde and acetic anhydride upon acetyl-glycine.⁹⁶



In like manner, α -bromopropionylalanine is converted into the azlactone of α -propionaminoacrylic acid, which on hydrolysis readily yields pyruvic acid; a similar series of reactions is undergone by α -bromopropionylasparagine.⁹⁷

The conversion of chloroacetamino acids to unsaturated azlactones

⁹² Neuberg, *Biochem. Z.*, **13**, 305 (1908).

⁹³ Lieben and Urban, *ibid.*, **239**, 250 (1931).

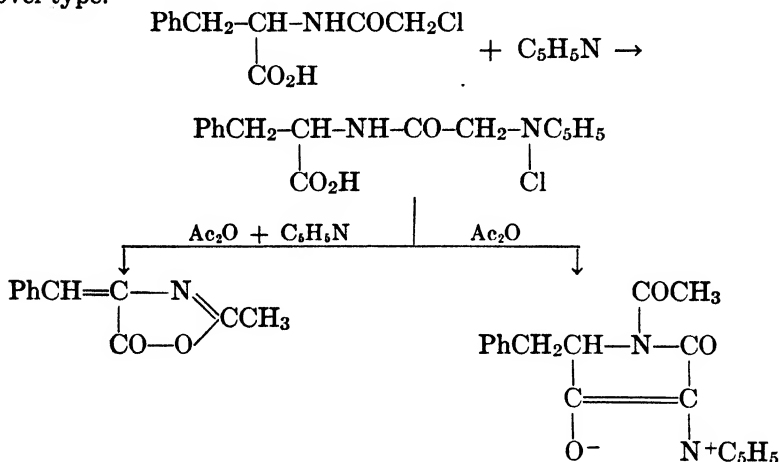
⁹⁴ Krebs, *Z. physiol. Chem.*, **217**, 191; **218**, 157 (1933); *Biochem. J.*, **29**, 1620 (1935).

⁹⁵ Bergmann and Stern, *Ann.*, **448**, 20 (1926).

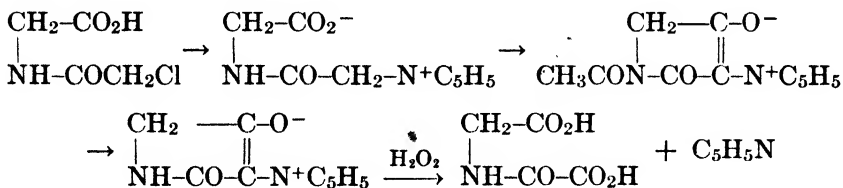
⁹⁶ Erlenmeyer and Früstück, *Ann.*, **284**, 36 (1895).

⁹⁷ Bergmann, Kann, and Miekeley, *Ann.*, **449**, 135 (1926).

proceeds even more readily in the presence of pyridine.⁹⁸ With pyridine alone a betaine hydrochloride is formed; when this is treated with a mixture of acetic anhydride and pyridine, it yields the azlactone; but with acetic anhydride alone, it is converted into a cyclic compound of a novel type.



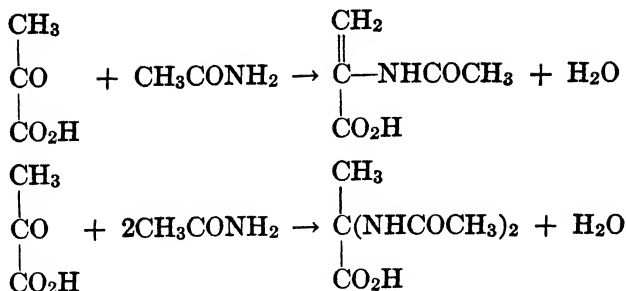
A similar product is formed by the action of a mixture of acetic anhydride and pyridine upon chloroacetyl glycine, which is of course incapable of yielding an unsaturated azlactone. These substances lose acetyl on heating with acid or alkali, but the pyridine can be regenerated only by pyrolysis or by disruptive oxidation.



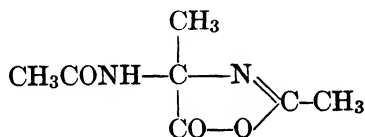
α,β -Unsaturated amino acids *per se* are apparently incapable of independent existence, but the presence of an acyl group on the nitrogen atom imparts sufficient stability to the molecule. A reversal of the effect of hydrolysis, mentioned above, can be brought about by condensing α -keto acids with acid amides. When, for instance, pyruvic acid is heated with acetamide, a mixture of α -acetaminopropionic acid and α,α -diacetaminopropionic acid results.⁹⁹

⁹⁸ Bergmann, Zervas, and Lebrecht, *Ber.*, **64**, 2315 (1931).

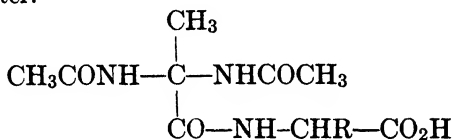
⁹⁹ Bergmann and Grafe, *Z. physiol. Chem.*, **187**, 187, 196 (1930).



The diacetamino compound is readily converted into acetaminoacrylic acid by heating with acetic acid. With acetic anhydride it yields an azlactone,

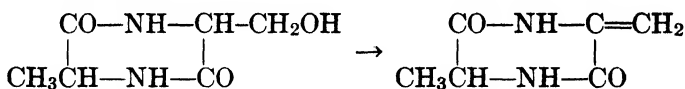


which readily reacts with amino acids to yield condensation products of peptide character.

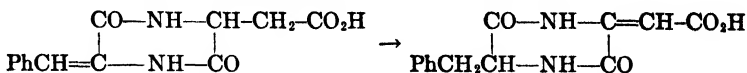


Acetaminoacrylic acid takes up hydrogen in presence of palladium, yielding *dl*-acetylalanine.

Derivatives of unsaturated amino acids can be produced by dehydration of diketopiperazines from α -amino- β -hydroxy acids.



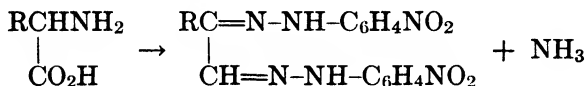
Derivatives of cystine can under suitable conditions lose the elements of hydrogen disulfide to yield the unsaturated compounds. In diketopiperazines derived from one saturated and one unsaturated amino acid, migration of the double bond is possible, so that in effect the unsaturated amino acid can dehydrogenate the saturated one.¹⁰⁰



¹⁰⁰ Bergmann and collaborators, *ibid.*, **146**, 247 (1925); **152**, 189 (1926); **174**, 76 (1928); *Ann.*, **458**, 40, 76 (1927).

This process is of especial interest as it constitutes a synthetic analogy of transformations which may take place metabolically in the living body.

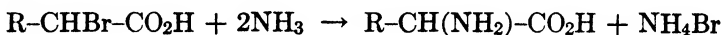
When an α -amino acid is digested in weakly acid solution with *p*-nitrophenylhydrazine, the bis-nitrophenylhydrazone of the corresponding α -ketoaldehyde is gradually deposited.¹⁰¹



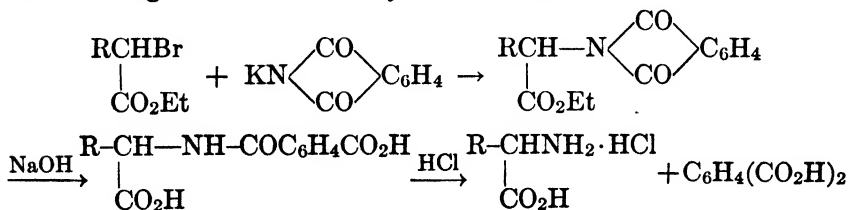
This remarkable reaction, for which no adequate theoretical explanation is available, takes place not only with α -amino acids in general but also with α -hydroxy acids. It is of peculiar interest to the biochemist inasmuch as it constitutes a reversal of a biological synthesis of amino acids¹⁰² in which a mixture of an α -ketonic aldehyde and ammonium carbonate, when perfused through a dog's liver, is converted into the corresponding amino acid. The reaction with α -hydroxy acids is likewise a reversal of the well-recognized enzymatic conversion of methylglyoxal to lactic acid.

GENERAL SYNTHETIC METHODS FOR PREPARING α -AMINO ACIDS

The classical method consisting in the action of ammonia upon α -halogen-substituted acids,



a recent example of which is the preparation of glycine,¹⁰³ has found the widest application. A modification of this principle, occasionally employed, involves the application of the phthalimide reaction¹⁰⁴ to esters of halogenated acids. The product is hydrolyzed with alkali, and the resulting acid heated with hydrochloric acid.



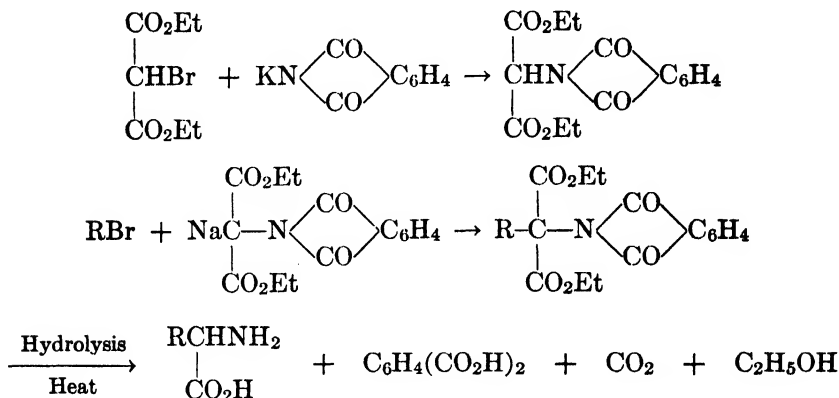
¹⁰¹ Dakin and Dudley, *J. Biol. Chem.*, **14**, 555; **15**, 127 (1913); Dakin, *Biochem. J.*, **10**, 313 (1916).

¹⁰² Dakin and Dudley, *J. Biol. Chem.*, **18**, 29 (1914).

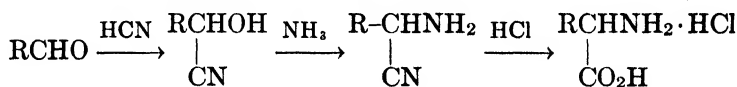
¹⁰³ Orten and Hill, *J. Am. Chem. Soc.*, **53**, 2797 (1931).

¹⁰⁴ Gabriel and Kroseberg, *Ber.*, **22**, 426 (1889).

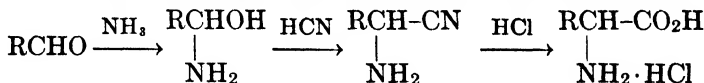
A general and extremely useful combination of the phthalimide and malonic ester synthesis has been developed by Sørensen.¹⁰⁵



The Strecker synthesis¹⁰⁶ from aldehydes (or ketones), ammonia, and hydrogen cyanide is of wide application in a variety of forms, all of which involve the intermediate formation of aminonitriles: (1) by the interaction of a cyanohydrin and ammonia;¹⁰⁷



(2) by the action of hydrogen cyanide on an aldehyde-ammonia;



or (3) by treating an aldehyde with ammonium cyanide, produced either by direct union of ammonia and hydrogen cyanide or by mixing concentrated solutions of potassium cyanide and ammonium chloride.

Modifications of the Strecker process, whereby hydantoins are formed directly by the use of ammonium carbonate, have recently been developed.¹⁰⁸ Hydantoins, which break down to amino acids on hydrolysis under vigorous conditions, can, owing to their lack of dipolar properties, often be more readily isolated than the corresponding amino acids.

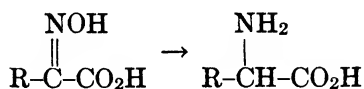
¹⁰⁵ Sørensen, *Z. physiol. Chem.*, **44**, 448 (1905).

¹⁰⁶ Strecker, *Ann.*, **75**, 27 (1850); Tiemann, *Ber.*, **13**, 381 (1880); **14**, 1965 (1881).

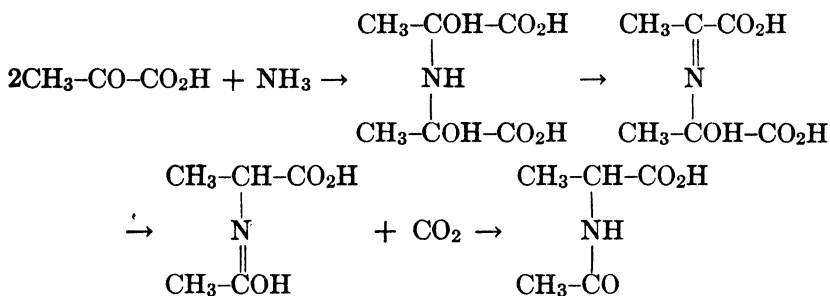
¹⁰⁷ Menge, *J. Am. Chem. Soc.*, **56**, 2197 (1934).

¹⁰⁸ Bucherer and collaborators, *J. prakt. Chem.*, **140**, 291; **141**, 5 (1934); Slotta, Behnisch, and Szyska, *Ber.*, **67**, 1529 (1934).

α -Amino acids have also been prepared by the reduction of α -oximino acids.¹⁰⁹

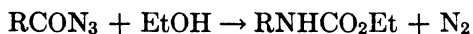


By this reaction it is possible to regenerate amino acids from the keto acids which constitute the primary products of their oxidative deamination. Of interest in this connection is the formation of acetylalanine by the action of ammonia upon pyruvic acid;¹¹⁰

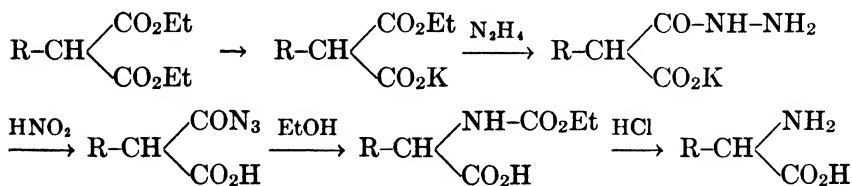


and phenacetylphenylalanine from ammonia and phenylpyruvic acid.

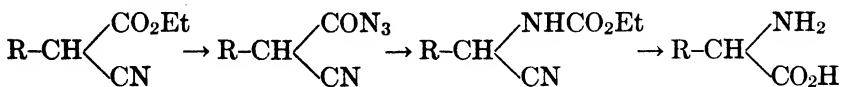
The decomposition of acid azides by alcohol, whereby urethanes are formed,



has been applied¹¹¹ to the preparation of α -amino acids from malonic esters;



and from cyanoacetic esters.¹¹²



In syntheses of higher or more complex amino acids, particularly those containing a terminal aromatic group, a derivative of glycine has

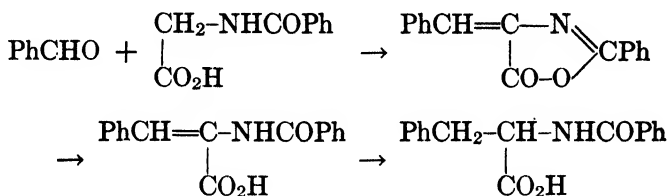
¹⁰⁹ Gutknecht, *Ber.*, **13**, 1116 (1880).

¹¹⁰ de Jong, *Rec. trav. chim.*, **19**, 259 (1900); Erlenmeyer, *Ann.*, **337**, 205 (1904).

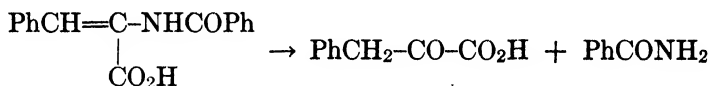
¹¹¹ Curtius, *J. prakt. Chem.*, [2] **135**, 211 (1930).

¹¹² Darapsky, *ibid.*, [2] **146**, 250 (1936).

in many instances been employed as starting material. Benzaldehyde may be condensed with hippuric acid in presence of acetic anhydride¹¹³ as in Perkin's synthesis, yielding the azlactone of benzoyl- α -amino-cinnamic acid, which on mild hydrolysis, followed by reduction with sodium amalgam, yields benzoylphenylalanine.

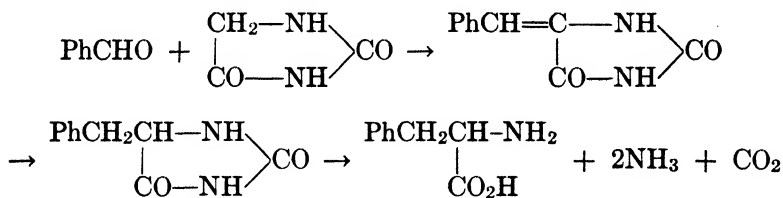


This is readily split by acid or by alkaline hydrolysis into *dl*-phenylalanine and benzoic acid. On subjecting the benzoylaminocinnamic acid to drastic hydrolysis, it breaks down into phenylpyruvic acid,



the oxime of which, on reduction, also yields phenylalanine.¹¹⁴ Acetyl-glycine or even glycine itself may conveniently be employed.^{95, 96, 115}

Similar syntheses have been carried out with hydantoin.¹¹⁶



The last two steps (reduction and hydrolysis) can conveniently be carried out in one operation by treating the condensation product with ammonium sulfide at 58°. ¹¹⁷ Acetylthiohydantoin may advantageously be employed in place of hydantoin.

An interesting synthesis involves the condensation of aromatic aldehydes with rhodanine.¹¹⁸ The product on treatment with alkali breaks

¹¹³ Erlenmeyer, *Ann.*, **275**, 1 (1893).

¹¹⁴ Erlenmeyer, *Ann.*, **271**, 137 (1892).

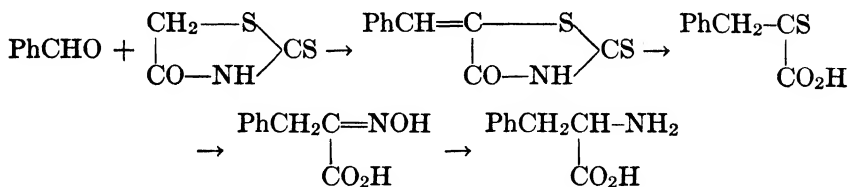
¹¹⁵ Dakin, *J. Biol. Chem.*, **82**, 439 (1929).

¹¹⁶ Wheeler and Hoffman, *Am. Chem. J.*, **45**, 368 (1911).

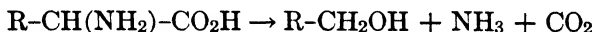
¹¹⁷ Boyd and Robson, *Biochem. J.*, **29**, 542, 546 (1935).

¹¹⁸ Gränacher, *Helv. Chim. Acta*, **5**, 610 (1922); **6**, 458 (1923).

down to an α -thioketo acid, which with hydroxylamine yields the corresponding oximino acid; this is then reduced.



All the above processes lead to inactive amino acids. Resolution has generally been effected by acylating the amino group and fractionally crystallizing the salts of the resulting acid with an optically active base such as an alkaloid. An alternative method recently developed¹¹⁹ consists in introducing the *l*-menthoxyacetyl group and separating the resulting mixture of diastereoisomeric acids by crystallization. The "unnatural" (*d*) varieties of amino acids can be prepared by subjecting the racemic forms to the action of actively fermenting sugar solutions,¹²⁰ whereby amino acids having the *l* configuration are converted, by reductive deamination and decarboxylation, into alcohols,

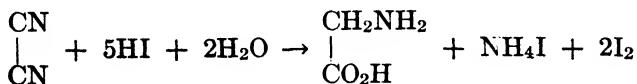


the *d*-amino acids remaining intact.

SOME INDIVIDUAL AMINO ACIDS AND DERIVATIVES

The subsequent pages contain discussion of the properties of all natural amino acids of protein origin except alanine, valine, leucine, isoleucine, norleucine, and phenylalanine. These compounds are all of great interest from the biochemical standpoint, but as they conform closely to the general type of monoamino monocarboxylic acid, reviewed above and treated in more detail in the case of glycine, their intimate discussion has not been undertaken.

Glycine. In addition to the general synthetic methods, outlined above, some special reactions have led to the formation of glycine. Cyanogen is simultaneously reduced and hydrolyzed by hot hydriodic acid.¹²¹

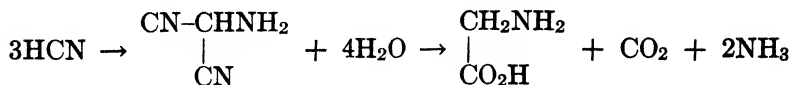


¹¹⁹ Holmes and Adams, *J. Am. Chem. Soc.*, **56**, 2093 (1934).

¹²⁰ Ehrlich, *Biochem. Z.*, **1**, 8 (1906); **8**, 438 (1908).

¹²¹ Emmerling, *Ber.*, **6**, 1351 (1873).

Hydrogen cyanide on long standing in presence of moisture is converted into a crystalline polymer $C_3H_3N_3$ which on boiling with acids or alkalis breaks down into glycine.¹²²

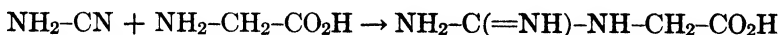


Formylglycine is formed by the action of ammonia upon glyoxylic acid¹²³ by a reaction analogous to that which occurs with pyruvic acid (p. 887).

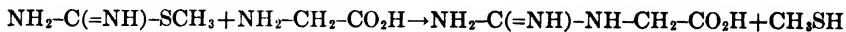
As already stated, glycine is oxidized by peroxides, catalyzed oxygen, or silver oxide to ammonia, carbon dioxide, and formaldehyde; on the other hand, it yields derivatives of oxalic acid on treatment with permanganate. The formation of ammonium oxamate, $\text{NH}_2\text{CO}-\text{CO}_2\text{NH}_4$, by the action of calcium permanganate upon gelatin¹²⁴ is ascribed to the presence of glycine combined in the protein.

The detection and estimation of glycine in a protein hydrolysate have generally been effected by taking advantage of the sparing solubility of its ethyl ester hydrochloride¹²⁵ or of its picrate.¹²⁶ The selective precipitation of a complex potassium trioxalatochromiate, $[\text{Cr}(\text{C}_2\text{O}_4)_3]_6\text{K}_{13}(\text{NH}_2\text{CH}_2\text{CO}_2\text{H})_5 \cdot 2\text{H}_2\text{O}$, has recently been suggested as a method for the quantitative isolation of glycine.¹²⁷ None of the other natural amino acids is precipitated under the conditions adopted. The analogous reagents in which the chromium is replaced by iron or cobalt are also selective precipitants for glycine.

On treatment with cyanamide, glycine is converted into guanidinoacetic acid, or glycoeyamine,



which is also formed by heating glycine with guanidine, or, more conveniently, S-methylisothiurea.¹²⁸



On boiling with dilute hydrochloric acid, glycoeyamine undergoes internal condensation to glycoeyamidine.¹²⁹

¹²² Lange, *Ber.*, **6**, 99 (1873); Wippermann, *Ber.*, **7**, 767 (1874).

¹²³ Erlenmeyer and Kunlin, *Ber.*, **35**, 2438 (1902).

¹²⁴ Kutscher and Schenck, *Ber.*, **37**, 2928 (1904).

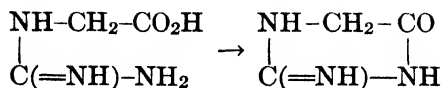
¹²⁵ Fischer and Skita, *Z. physiol. Chem.*, **33**, 177 (1901); Fischer, *ibid.*, **35**, 227 (1902).

¹²⁶ Levene, *J. Biol. Chem.*, **1**, 413 (1906); Levene and Van Slyke, *ibid.*, **12**, 285 (1912).

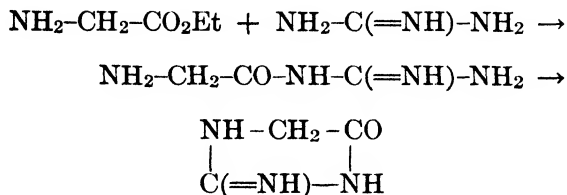
¹²⁷ Bergmann and Fox, *ibid.*, **109**, 317 (1935).

¹²⁸ Nencki and Sieber, *J. prakt. Chem.*, [2] **17**, 477 (1878). Wheeler and Merriam, *Am. Chem. J.*, **29**, 478 (1903).

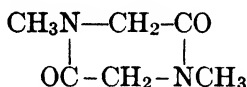
¹²⁹ Jaffé, *Z. physiol. Chem.*, **48**, 430 (1906).



This compound is also formed by the action of guanidine upon glycine ester.¹³⁰

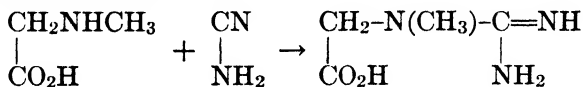


Sarcosine, the N-methyl derivative of glycine, has not been found among the hydrolytic products of proteins, but is of interest in that it is formed by the alkaline hydrolysis of caffeine and creatine. It has been synthesized from ethyl chloroacetate and methylamine, by the action of formaldehyde and tin upon glycine in boiling acid,⁶¹ and from benzene-sulfonylglycine and methyl sulfate.¹³¹ In its general properties it closely resembles glycine. On heating above 200° it breaks down, partly into carbon dioxide and dimethylamine and partly into water and the diketopiperazine, sarcosine anhydride.¹³²



It is oxidized more rapidly than glycine by silver oxide (*cf.* p. 881), yielding carbon dioxide, formaldehyde, and methylamine.

Creatine, an important constituent of muscle extract and of certain biological fluids, is α -methylguanidinoacetic acid; it has been synthesized from sarcosine and cyanamide,



or (together with creatinine) from sarcosine and guanidine carbonate.¹³³

Guanidine and its alkyl derivatives are very strong bases, being as highly dissociated in aqueous solution as potassium hydroxide,*¹³⁴

¹³⁰ Traube and Ascher, *Ber.*, **46**, 2077 (1913).

¹³¹ Cocker and Lapworth, *J. Chem. Soc.*, 1894 (1931).

¹³² Mylius, *Ber.*, **17**, 286 (1884).

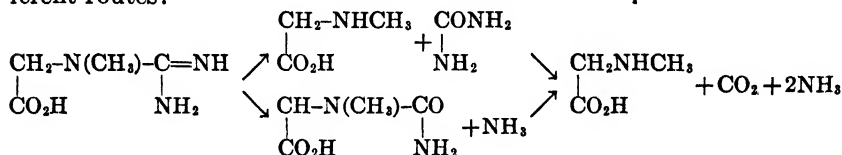
¹³³ Volhard, *Jahresb.*, 685 (1868); Paulmann, *Arch. Pharm.*, **232**, 601 (1894).

¹³⁴ Davis and Elderfield, *J. Am. Chem. Soc.*, **54**, 1499 (1932).

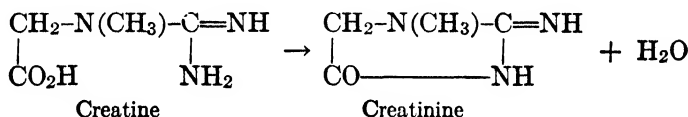
* Symmetrical dialkylguanidines, of which the *pK* is only about 10.2, form an exception to this generalization.

creatine accordingly possesses dipolar properties which are even more pronounced than those of the amino acids.¹³⁵ From determinations of its dissociation constant in acid solution¹³⁶ and its solubility in alkaline solutions,¹³⁷ it has been estimated that creatine is a thousandfold stronger as a base than as an acid. Its solubility in cold water is 1.5 per cent; in solution it is neutral to litmus, and undoubtedly consists mainly of dipolar ions.

In boiling alkaline solution it breaks down into sarcosine, carbonic acid, and ammonia. The decomposition¹³⁸ proceeds along two different routes:



In acid solution, on the other hand, ring closure occurs, as in the hydantonic acids (p. 874), with formation of creatinine.



On treatment with alcoholic hydrogen chloride, creatine yields ester hydrochlorides.¹³⁹ The free esters, which should be extremely strong bases, appear to be incapable of independent existence, for when the hydrochloric acid is removed, alcohol is simultaneously split off, with formation of creatinine. This loss of alcohol also takes place merely on heating the hydrochloride alone or with water—so readily, indeed, that creatine ester hydrochlorides have been regarded as salts of creatinine in which alcohols are bound in some undetermined manner. However, titration curves of creatine ester salts not only clearly demonstrate the difference of the dissociation characteristics of the ester from those of creatinine but also indicate an irreversible conversion of ester to creatinine during the progress of the titration from pH 3.5 to pH 5.5. The readiness with which this ring closure takes place is not without parallel:

¹³⁵ Cannan and Shore, *Biochem. J.*, **22**, 920 (1928).

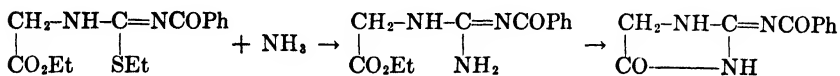
¹³⁶ Hahn and Barkan, *Z. Biol.*, **72**, 25 (1920); Eadie and Hunter, *J. Biol. Chem.*, **67**, 237 (1926).

¹³⁷ Hahn and Fasold, *Z. Biol.*, **82**, 473 (1925).

¹³⁸ Gaebler, *J. Biol. Chem.*, **69**, 613 (1926).

¹³⁹ Dox and Yoder, *ibid.*, **54**, 671 (1922). Kapfhammer, *Biochem. Z.*, **156**, 182 (1925). Failey and Brand, *J. Biol. Chem.*, **102**, 767 (1933).

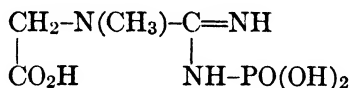
on treating benzoylpseudoethylthiohydantoic ester with ammonia, the resulting ethyl ester of benzoylglycocyanine¹⁴⁰ spontaneously loses alcohol, at the moment of formation, to yield benzoylglycocyanidine.



Analogous cases are cited on pp. 898, 928 and 930.

On treatment with acetic anhydride, creatine is converted into a diacetyl derivative.¹⁴¹ An ester of diacetylcreatine has been produced by means of an interesting reaction discovered by Bergmann and Zervas (p. 928).

Of great biochemical interest is creatine-phosphoric acid, or phosphocreatine,



an unstable constituent of mammalian muscle tissue.¹⁴² The hydrolytic breakdown of this substance into creatine and phosphoric acid, which occurs under the influence of enzymes present in muscle, is exothermic and is associated with the development of muscular energy; regeneration occurs during repose.

Creatinine, which is formed from creatine by the action of mineral acids, is more soluble in water and, being more electrically unbalanced, behaves as a stronger base than creatine.^{136, 143} In aqueous solution, creatine and creatinine enter into equilibrium, the stationary state being established very slowly in the cold but more rapidly at higher temperatures. The reaction rate also depends on the pH level, reaching a maximum at pH 4.¹³⁵ The composition of the equilibrium mixture depends upon the pH of the solution, the ratio of creatinine to creatine rising rapidly from 2 at pH 4 to 20 at pH 2. At pH 5 to 7, the components are present in approximately equimolar ratio.¹⁴⁴ In more strongly alkaline solution, hydrolysis to ammonia, methylhydantoin, urea, and sarcosine occurs.¹³⁸

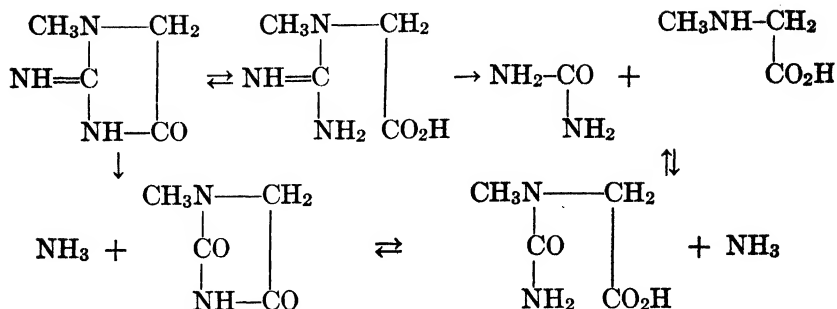
¹⁴⁰ Johnson and Nicolet, *J. Am. Chem. Soc.*, **37**, 2416 (1915).

¹⁴¹ Erlenmeyer, *Ann.*, **284**, 49 (1895).

¹⁴² Eggleton and Eggleton, *Biochem. J.*, **21**, 190 (1927); Meyerhof and Lohmann, *Biochem. Z.*, **196**, 22, 49 (1928); Fiske and Subbarow, *J. Biol. Chem.*, **81**, 629 (1929); Parnas and Ostern, *Biochem. Z.*, **279**, 94 (1935).

¹⁴³ McNally, *J. Am. Chem. Soc.*, **48**, 1003 (1926).

¹⁴⁴ Edgar and Shiver, *ibid.*, **47**, 1179 (1925).



Creatinine forms a characteristic picrate which is sparingly soluble in water. On the addition of alkali, this yellow picrate develops an orange-red color. Creatine also forms a picrate which closely resembles that of creatinine but yields merely a yellow solution on treatment with alkali. These observations, recorded by Jaffé, form the basis of an analytical method for the estimation of creatinine.¹⁴⁵ The development of the red color in this test is not specific for creatinine, and has been observed with glycoeyamidine, hydantoins, barbituric acid, and diketopiperazines. On the other hand, no color is formed by derivatives of creatinine in which both of the imino hydrogen atoms are replaced by methylol groups, or the methylene hydrogen atoms by benzylidene. The red color was long supposed to be due to picramic acid, which is formed from picric acid by a variety of reducing substances; it now appears to be caused by the formation of a salt of a red tautomer of creatinine picrate.¹⁴⁶ Picric acid seems to be essential for the Jaffé reaction, which does not occur with 2,4- and 2,6-dinitrophenols nor even with 2,4,6-trinitro-m-cresol.

Creatinine is capable of forming a silver derivative which contains one atom of the metal. On treatment with alkyl iodides, as many as three alkyl groups can be introduced, the third involving the formation of a quaternary ammonium salt.¹⁴⁷

Dimethylglycine can be prepared by the interaction of chloroacetic acid and dimethylamine; by condensing formaldehyde cyanohydrin with dimethylamine and then hydrolyzing;¹⁴⁸ also, by treating glycine with formaldehyde in the presence of tin and hydrochloric acid,⁶¹ or of formic

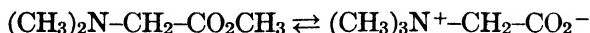
¹⁴⁵ Jaffé, *Z. physiol. Chem.*, **10**, 391 (1886); Folin, *ibid.*, **41**, 223 (1904); *J. Biol. Chem.*, **17**, 463, 469, 475 (1914); Folin and Doisy, *ibid.*, **28**, 349 (1917).

¹⁴⁶ Greenwald and Gross, *ibid.*, **59**, 601 (1924). Greenwald, *J. Am. Chem. Soc.*, **47**, 1443 (1925); *J. Biol. Chem.*, **77**, 539; **80**, 103 (1928); **86**, 333 (1930); Analow and King, *J. Chem. Soc.*, 1210 (1929).

¹⁴⁷ Schmidt, *Arch. Pharm.*, **248**, 568 (1910); Kunze, *ibid.*, **248**, 578; Henzlerling, *ibid.*, **248**, 594 (1910).

¹⁴⁸ Eschweiler, *Ann.*, **279**, 39 (1894).

acid.⁶³ The methyl ester, when heated, rearranges reversibly into betaine,



an example of the alkylating action of carboxylic esters.¹⁴⁹

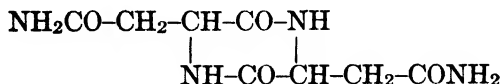
Betaine salts such as the hydrochloride, which can be titrated as mono-carboxylic acids, are formed by the addition of chloroacetic acid to trimethylamine or by treating glycine or sarcosine with methyl iodide or sulfate. At 260–270° betaine hydrochloride breaks down into tetramethylammonium chloride and carbon dioxide.

Esters of betaine are of course capable of existing only in the form of salts; these are formed directly from trimethylamine and chloroacetic esters, from methyl iodide and an ester of dimethylglycine, or as a by-product in the methylation of glycine.¹⁵⁰ On treatment with ammonia they are converted into salts of betaine-amide. The corresponding hydrazide,¹⁵¹ similarly produced from hydrazine, has recently found application as a reagent for preparing water-soluble derivatives of insoluble ketones such as sex hormones.

Aspartic and Glutamic Acids. The chemical character of the monoamino dicarboxylic acids resembles in the main that of the mono-carboxylic acids; such differences as exist are ascribable to the presence of the second carboxyl group.

Both acids are less soluble in water than the corresponding mono-carboxylic acids; the *pH* of their aqueous solutions is low, but higher than the isoelectric point (cf. p. 867). They are extracted by butyl alcohol from their solutions at *pH* 3.¹⁶ Both form sparingly soluble salts with heavy metals; the salts of barium and calcium are insoluble in alcohol.¹⁵²

dl-Aspartic acid is formed by direct addition of ammonia to maleic or fumaric acid.¹⁵³ Ethyl fumarate reacts with ammonia to form diethyl aspartate¹⁵⁴ and diketopiperazine diacetamide,¹⁵⁵



¹⁴⁹ Willstätter and collaborators, *Ber.*, **35**, 584, 2757 (1902); Hammett and Pfluger, *J. Am. Chem. Soc.*, **55**, 4079 (1933).

¹⁵⁰ Novak, *Ber.*, **45**, 834 (1912).

¹⁵¹ Girard and Sandulesco, *Helv. Chim. Acta*, **19**, 1095 (1936).

¹⁵² Foreman, *Biochem. J.*, **8**, 461, 481 (1914).

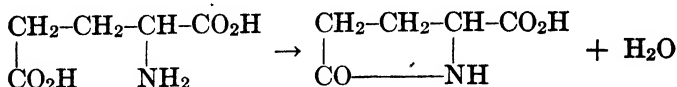
¹⁵³ Engel, *Bull. soc. chim.*, [2] **48**, 97 (1887); **50**, 149 (1888).

¹⁵⁴ Koerner and Menozzi, *Gazz. chim. ital.*, **17**, 220 (1887).

¹⁵⁵ Fischer and Koenigs, *Ber.*, **37**, 4585 (1904).

which on alkaline hydrolysis is converted into *dl*-aspartic acid.¹⁵⁶ Syntheses of glutamic acid have followed more conventional lines.

An important difference between aspartic and glutamic acids is represented by the readiness with which the latter passes over into pyrrolidonecarboxylic acid in hot aqueous solution.¹⁵²



This reaction, which is reversed by the action of hot concentrated hydrochloric acid, finds no analogy in the case of aspartic acid, and is of course referable to the spatial proximity, in glutamic acid, of the groups involved.

Another difference, probably due to a similar cause, resides in the contrasting stabilities of the naturally occurring monoamides of the two acids, both of which are widely distributed in growing vegetation and have been isolated from enzymatic digests of vegetable proteins.¹⁵⁷ Asparagine, $\text{NH}_2\text{COCH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$, is relatively stable in aqueous solution over a wide range of hydrogen-ion concentration. Glutamine, $\text{NH}_2\text{COCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$, rapidly undergoes hydrolysis to ammonium pyrrolidonecarboxylate¹⁵⁸ in neutral solution at 100° , under which conditions asparagine is hardly affected. Introduction of an aminoacyl group into glutamine stabilizes the amide linkage, possibly by reducing the acidic dissociation of the neighboring carboxyl group.

Glutamine, readily preparable from the common beet,¹⁵⁹ appears to be intimately involved in the detoxification of ammonia and the assimilation of nitrogen by plants and animals. Vegetable organisms grown under conditions in which ammonia is the sole source of nitrogen accumulate relatively large proportions of glutamine,¹⁶⁰ which is also synthesized in kidney and other tissues from *l*(+)-glutamic acid and ammonia.¹⁶¹ This synthesis is an endothermic process, and absorbs energy from other biochemical reactions; when formed in brain tissue, glutamine is converted to other substances of unknown character, without liberation of ammonia. These changes do not take place with *d*(-)-glutamic acid. Mammalian tissues also contain at least two

¹⁵⁶ Dunn and Fox, *J. Biol. Chem.*, **101**, 493 (1933).

¹⁵⁷ Damodaran and collaborators, *Biochem. J.*, **26**, 235, 1704 (1932).

¹⁵⁸ Vickery and collaborators, *ibid.*, **29**, 2710 (1935).

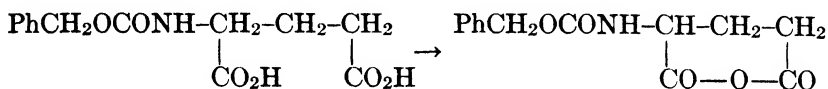
¹⁵⁹ Vickery, Pucher and Clark, *J. Biol. Chem.*, **109**, 39 (1935).

¹⁶⁰ Greenhill and Chibnall, *Biochem. J.*, **28**, 1422 (1934); Vickery and collaborators, *Science*, **80**, 459 (1934); *J. Biol. Chem.*, **113**, 157 (1936); *Plant Physiol.*, **11**, 413 (1936).

¹⁶¹ Krebs, *Biochem. J.*, **29**, 1951 (1935).

enzymes (glutaminases) which cause the hydrolysis of glutamine to ammonium glutamate. One of these, present in liver, operates only at high hydroxyl-ion concentrations; the other, present in brain, acts in the physiological range of pH .

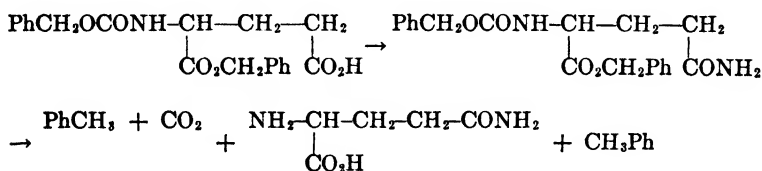
The synthetic conversion of $l(+)$ -glutamic acid into the natural variety of glutamine exemplifies some of the elegant preparative methods developed by Bergmann.¹⁶² On treatment with benzyl chlorocarbonate, glutamic acid yields the carbobenzoxy derivative, which with acetic anhydride is transformed into an anhydride.



This on treatment with ammonia yields a carbobenzoxy derivative of iso-glutamine,¹⁶²



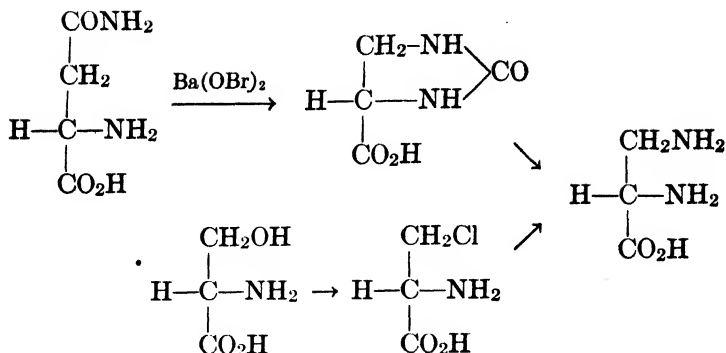
isomeric with that from natural glutamine. A similar reaction occurs with benzyl alcohol, the α -monobenzyloxy ester being the sole product. This is converted, through the chloride, into the amide; when this compound is catalytically hydrogenated, the benzyl groups are split off as toluene and the resulting unstable carbamic acid grouping loses carbon dioxide, leaving glutamine.



Iso-glutamine is formed in the same way from its carbobenzoxy derivative. Aspartic acid may be converted into asparagine and iso-asparagine by an analogous series of reactions.

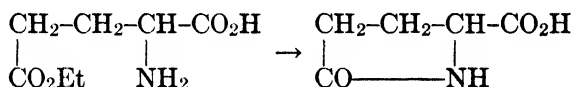
On treatment with hypobromite, followed by hydrolysis, l -asparagine is converted into an optically active α,β -diaminopropionic acid identical with that prepared by the action of ammonia upon the β -chloroalanine derived from natural serine.

¹⁶² Bergmann and collaborators, *Z. physiol. Chem.*, **221**, 51 (1933); *Ber.*, **65**, 1192 (1932); **66**, 1288 (1933).

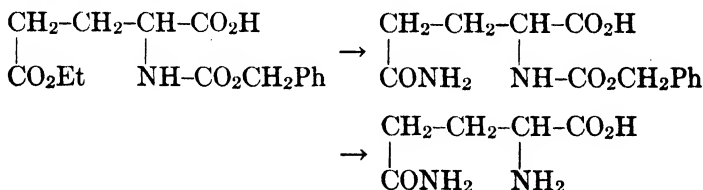


This series of reactions establishes the configurational identity of natural aspartic acid and serine. By a similar process, glutamine yields an α,γ -diaminobutyric acid which with its dibenzoyl esters displays optical relations analogous to those shown by the natural α,δ - and α,ϵ -diamino acids ornithine and lysine.¹⁶³

The γ -monoethyl ester of glutamic acid, prepared by the action of ethyl iodide upon silver glutamate and by direct esterification of glutamic acid, readily undergoes auto-condensation to pyrrolidonecarboxylic acid.



When its carbobenzoxy derivative is successively treated with ammonia and hydrogenated, glutamine results.¹⁶⁴



Proline. This widely distributed amino acid is distinguished from all others, except hydroxyproline, by its inability to yield nitrogen on treatment with nitrous acid and by its ready solubility in alcohol. The solubility of its copper salt in water, methyl alcohol, and absolute ethyl alcohol⁸ has been employed for the isolation of proline.¹⁶⁵ A more

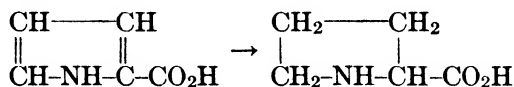
¹⁶³ Karrer and collaborators, *Helv. Chim. Acta*, **6**, 411, 957 (1923); **9**, 301 (1926).

¹⁶⁴ Nienburg, *Ber.*, **68**, 2232 (1935).

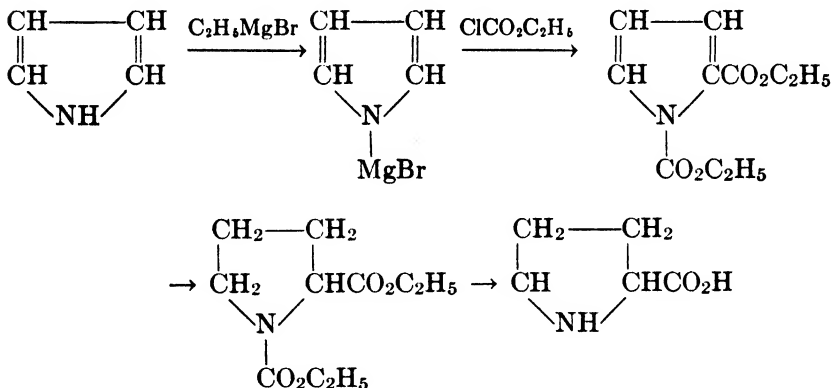
¹⁶⁵ Klabunde, *J. Biol. Chem.*, **90**, 293 (1931).

selective process¹⁶⁶ involves its precipitation (after the removal of histidine and arginine) as the rhodanilate by means of ammonium rhodanilate, $[\text{Cr}(\text{SCN})_4 \cdot (\text{PhNH}_2)_3]_2(\text{NH}_4)_2 \cdot 3\text{H}_2\text{O}$. Reinecke salt,¹⁶⁷ $[\text{Cr}(\text{SCN})_4 \cdot (\text{NH}_3)_2]\text{NH}_4 \cdot \text{H}_2\text{O}$, forms a similar precipitate with both proline and hydroxyproline.¹⁶⁸

Proline has been synthesized by several methods. The most obvious, the hydrogenation of α -pyrrolicarboxylic acid, proceeds with difficulty, but can be accomplished in acid alcoholic solution by means of platinum oxide activated by ferric chloride.¹⁶⁹



A more convenient process involves the hydrogenation, under high pressure in presence of Raney nickel, of the 1,2-dicarbethoxypyrrole obtained by the action of ethylmagnesium bromide and ethyl chloro-carbonate upon pyrrole.¹⁷⁰



Pyrrolidonecarboxylic ester yields proline on reduction with sodium and alcohol.¹⁷¹

Other syntheses start from trimethylene bromide with malonic ester;¹⁷²

¹⁶⁶ Bergmann, *ibid.*, **110**, 471 (1935).

¹⁶⁷ Dakin, "Organic Syntheses," Wiley and Sons, New York (1935), Vol. 15, p. 74.

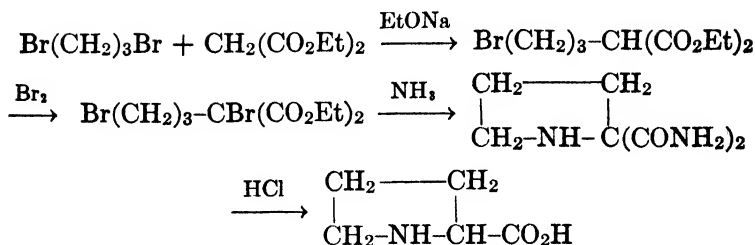
¹⁶⁸ Kapfhammer and collaborators, *Z. physiol. Chem.*, **170**, 294 (1927); **173**, 245 (1928).

¹⁶⁹ Putokhin, *J. Russ. Phys.-Chem. Soc.*, **62**, 2209 (1930) [*C. A.*, **25**, 3995 (1931)].

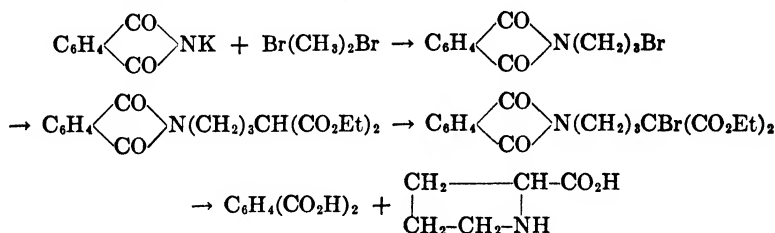
¹⁷⁰ Signaigo and Adkins, *J. Am. Chem. Soc.*, **58**, 1122 (1936).

¹⁷¹ Fischer and Bochner, *Ber.*, **44**, 1332 (1911).

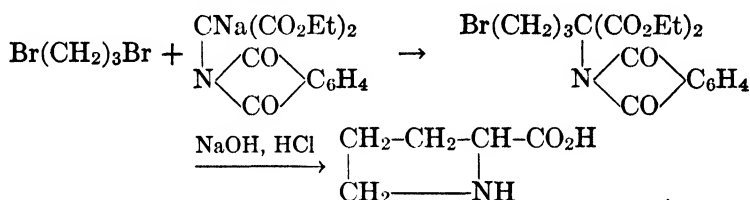
¹⁷² Willstätter and Ettlinger, *Ann.*, **326**, 91 (1903).



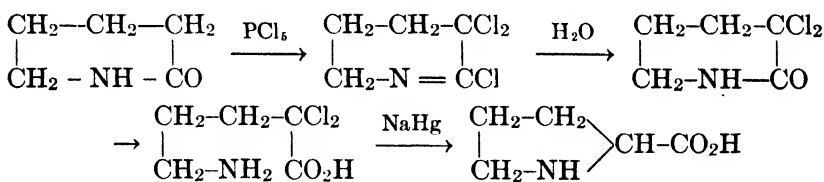
phthalimide and malonic ester;¹⁷³



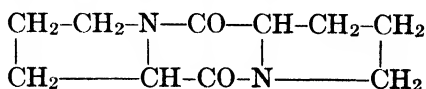
or phthaliminomalonic ester.¹⁷⁴



A synthesis of entirely different type starts from α -piperidone.¹⁷⁵



Like other amino acids, proline yields an ester hydrochloride on boiling with alcoholic hydrogen chloride. The free esters of proline are unstable and spontaneously lose the elements of alcohol to yield the tricyclic diketopiperazine, proline anhydride.¹⁷⁶



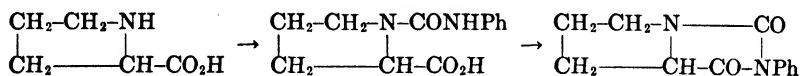
¹⁷³ Fischer, *Ber.*, **34**, 454 (1901).

¹⁷⁴ Sørensen and Andersen, *Z. physiol. Chem.*, **56**, 236 (1908).

¹⁷⁵ Heymons, *Ber.*, **66**, 846 (1933).

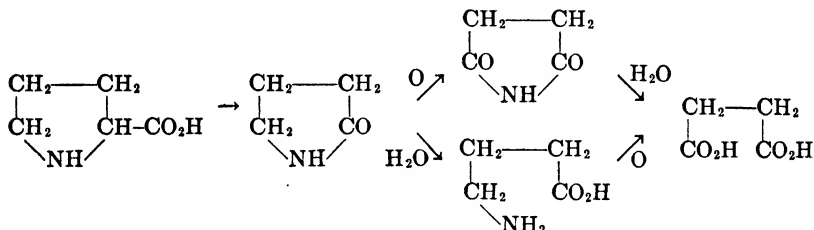
¹⁷⁶ Kapfhammer and Matthes, *Z. physiol. Chem.*, **223**, 43 (1934).

With phenyl isocyanate proline yields the corresponding hydantoic acid, which with hydrochloric acid is converted into the phenyl-hydantoin.¹⁷⁵



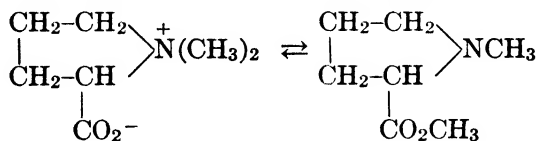
The conversion of proline into a carbamate by the action of carbon dioxide in presence of barium hydroxide (p. 874) has served⁵⁴ for its isolation from protein hydrolysates.

Electrolytic oxidation in acid solution¹⁷⁷ converts proline first into α -pyrrolidone, which then is partly hydrolyzed to γ -aminobutyric acid and partly further oxidized to succinimide.



Putrefactive organisms cause the reduction of proline to δ -aminovaleric acid and n -valeric acid.

On methylation, proline is converted into its betaine, stachydrine, the picrate, mercurichloride and aurichloride of which are sparingly soluble and afford a form in which proline can be quantitatively estimated.¹⁷⁸ Stachydrine, when heated under reduced pressure at 235°, passes over into the isomeric methyl ester of hygric acid.¹⁷⁹



Serine. Serine occurs in proteins of both animal and vegetable origin. It has been synthesized by applying the Strecker cyanhydrin procedure to glycollaldehyde and, more conveniently, to ethoxyacetaldehyde;¹⁸⁰ in the latter synthesis the final hydrolysis is effected with hydrobromic acid to remove the ethyl group. Serine has also been

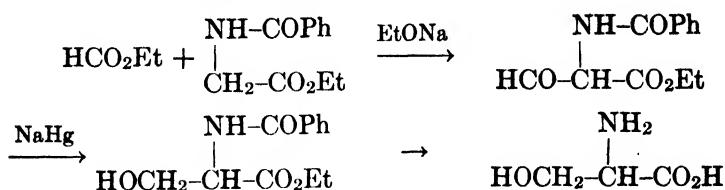
¹⁷⁷ Takayama, *Bull. Chem. Soc. Japan*, **11**, 138 (1936).

¹⁷⁸ Engeland, *Ber.*, **42**, 2962 (1909); *Z. physiol. Chem.*, **120**, 130 (1922).

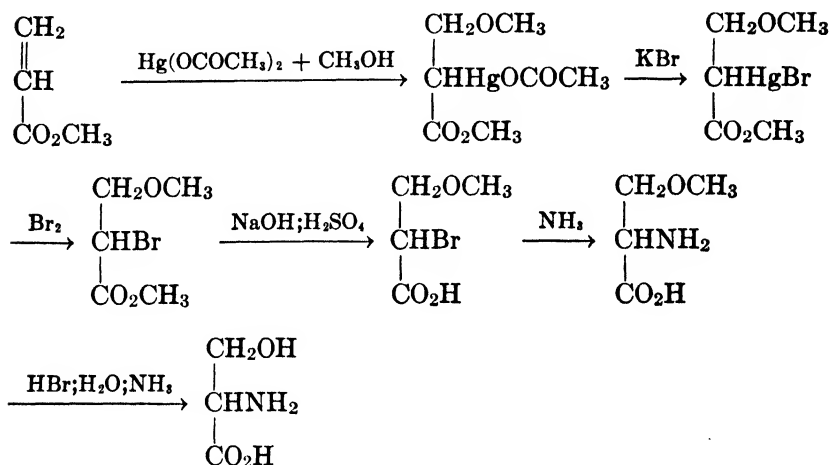
¹⁷⁹ Schulze and Trier, *ibid.*, **67**, 59 (1910); Trier, *ibid.*, **67**, 324 (1910).

¹⁸⁰ Dunn, Redemann and Smith, *J. Biol. Chem.*, **104**, 511 (1934).

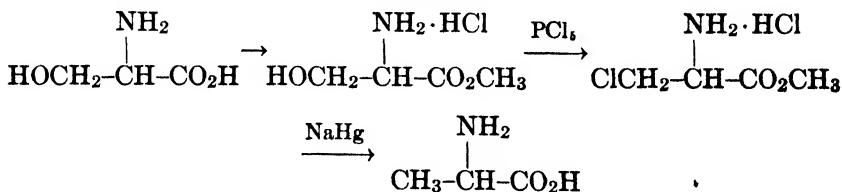
synthesized by the condensation of ethyl hippurate with ethyl formate, with subsequent reduction and hydrolysis,¹⁸¹



and from methyl acrylate¹⁸² by the following series of reactions:



The resolution of racemic serine has been effected by fractional crystallization of alkaloid salts of its *p*-nitrobenzoyl derivative.¹⁸³ The configurational relationship of the natural (−) variety to natural (+) alanine has been established¹⁸⁴ by direct conversion.



Racemic alanine is formed by heating inactive serine with hydriodic acid and phosphorus.

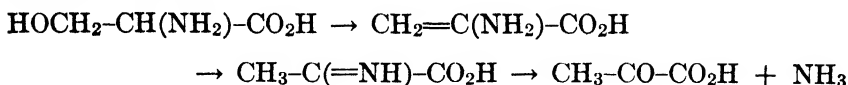
¹⁸¹ Erlenmeyer and Stoop, *Ann.*, **337**, 236 (1904).

¹⁸² Schiltz and Carter, *J. Biol. Chem.*, **116**, 793 (1936).

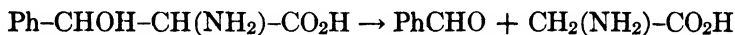
¹⁸³ Fischer and Jacobs, *Ber.*, **39**, 2942 (1906).

¹⁸⁴ Fischer and Raske, *Ber.*, **40**, 3717 (1907).

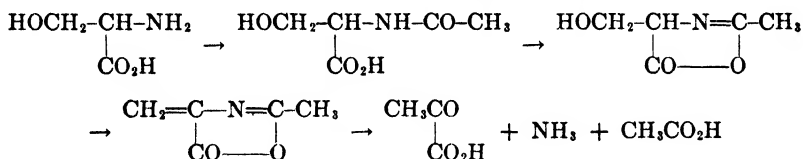
On boiling with sulfuric acid, serine slowly breaks down into ammonia and pyruvic acid.^{185, 186}



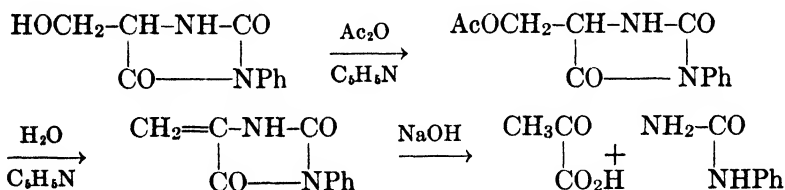
This change occurs more readily in alkaline solution¹⁸⁷ but is complicated by side reactions which lead to the production of oxalic acid, lactic acid, alanine, and glycine. The formation of glycine has been attributed¹⁸⁸ to a reaction analogous to reversal of aldolization, which takes place more smoothly with phenylserine.¹⁸⁶



The conversion of serine to pyruvic acid has also been effected by reactions in which intermediate products have been isolated;¹⁸⁹ either by dehydration and subsequent acid hydrolysis of an azlactone;



or by acetylation and alkaline hydrolysis of the phenylhydantoin.



The phenylhydantoin of serine can also be broken down directly into pyruvic acid and phenylurea by treatment with alkali.

On treatment with nitrous acid¹⁸⁶ or exposure to light in the presence of oxygen and a trace of ferrous sulfate¹⁹⁰ serine yields small quantities of acetaldehyde. The principal reaction with nitrous acid, however, is the normal formation of glyceric acid.

¹⁸⁵ Erlenmeyer, *Ber.*, **35**, 3769 (1902).

¹⁸⁶ Bettzieche, *Z. physiol. Chem.*, **150**, 177 (1925).

¹⁸⁷ Daft and Coghill, *J. Biol. Chem.*, **90**, 341 (1931).

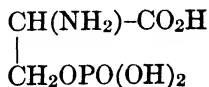
¹⁸⁸ Nicolet, *Science*, **74**, 250 (1931).

¹⁸⁹ Bergmann and Delis, *Ann.*, **458**, 76 (1927).

¹⁹⁰ Neuberg, *Biochem. Z.*, **67**, 59 (1914).

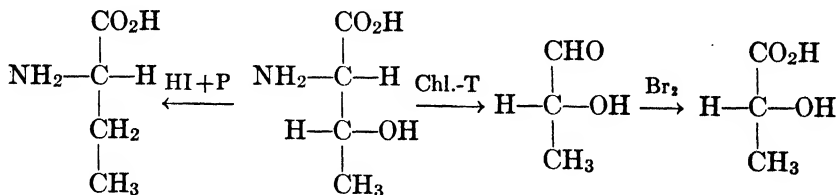
On subjection to putrefactive organisms serine yields propionic and formic acids¹⁹¹ or, under anaerobic conditions, ethanolamine.¹⁹²

A phosphorus-containing amino acid having the composition of serine-phosphoric acid,



has been isolated¹⁹³ from the products of acid hydrolysis of vitellic acid, a hydrolysis product of vitellin (from egg yolk), and of casein.¹⁹⁴ The compound, which is slowly hydrolyzed in acid, rapidly in alkaline solution, has been synthesized by the action of phosphorus pentoxide in phosphoric acid upon *dl*-serine, followed by resolution of the brucine salts.

Threonine. A homolog of serine, α -amino- β -hydroxybutyric acid, is a constituent amino acid of proteins. It was first isolated, in optically inactive form, from the monoamino monocarboxylic fraction by taking advantage of the solubility of its zinc salt in water and in alcohol and the insolubility of its copper salt in methyl alcohol.¹⁹⁵ Its isolation in optically active form has been accomplished by Rose and his collaborators,¹⁹⁶ who have demonstrated its indispensability for growth in the rat and have established its constitution. The configuration of the α -carbon atom is that of the natural (*l*) amino acids, since on reduction with hydriodic acid and phosphorus it yields the *l*(+)- α -amino-*n*-butyric acid identical with that obtained from biological material. On oxidation successively with Chloramine T and with bromine it is converted into *d*(-)-lactic acid.¹⁹⁷



The constitution thereby established is supported by the behavior with nitrous acid, which with α -amino acids brings about the replacement

¹⁹¹ Brasch, *ibid.*, **22**, 403 (1909).

¹⁹² Nord, *ibid.*, **95**, 281 (1919).

¹⁹³ Levene and collaborators, *J. Biol. Chem.*, **98**, 109 (1932); **103**, 537 (1933); **105**, 547; **106**, 595 (1934).

¹⁹⁴ Lipmann, *Biochem. Z.*, **262**, 3, 9 (1933).

¹⁹⁵ Schryver and Buston, *Proc. Roy. Soc. (London)*, **B99**, 476 (1926).

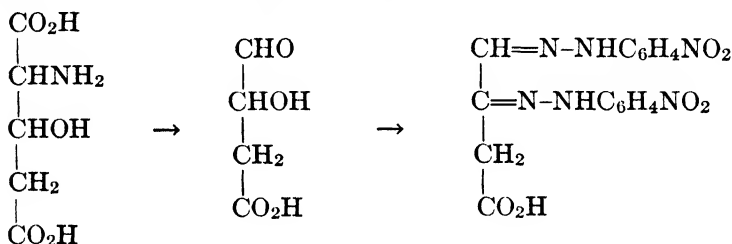
¹⁹⁶ McCoy, Meyer, and Rose, *J. Biol. Chem.*, **112**, 283 (1935).

¹⁹⁷ Meyer and Rose, *ibid.*, **115**, 721 (1936).

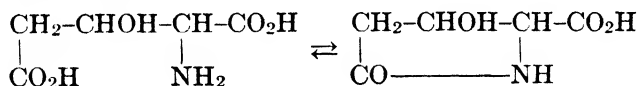
of amino by hydroxyl groups without Walden inversion:¹⁹⁸ the threo-dihydroxybutyric acid corresponding to *d*(-)-threose is produced. It is proposed that the natural α -amino- β -hydroxybutyric acid, although it is an *l*-amino acid, be termed *d*(-)-threonine.

β -Hydroxyglutamic Acid. The dicarboxylic acid fractions of hydrolyzed casein and zein have been shown by Dakin¹⁹⁹ to contain, besides aspartic and glutamic acid, a β -hydroxyglutamic acid, which appears to be a constituent of a phosphopeptone from casein.²⁰⁰ Owing to the technical difficulties involved in its isolation²⁰¹ this compound has received little attention, and no attempt has been made to ascertain the configuration of the β -carbon atom, which would be of especial interest in view of the close structural relationship to threonine.

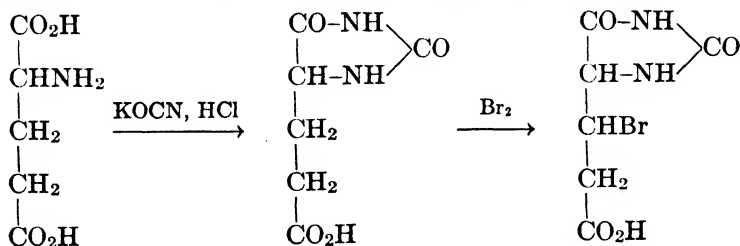
On successive treatment with Chloramine T and *p*-nitrophenylhydrazine it yields the osazone of malic semi-aldehyde.



In hot aqueous solution it passes over reversibly into a hydroxypyrrolidonecarboxylic acid.²⁰²



Two syntheses of inactive β -hydroxyglutamic acid have been devised. The first of these²⁰² starts with glutamic acid.



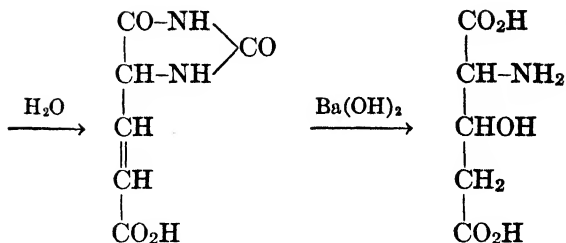
¹⁹⁸ Levene, *Chem. Rev.*, **2**, 179 (1926).

¹⁹⁹ Dakin, *Biochem. J.*, **12**, 290 (1918); *Z. physiol. Chem.*, **130**, 159 (1923).

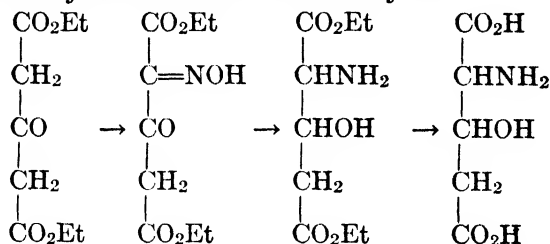
²⁰⁰ Rimington, *Biochem. J.*, **21**, 1179, 1187 (1927).

²⁰¹ Gulland and Morris, *J. Chem. Soc.*, 1644 (1934).

²⁰² Dakin, *Biochem. J.*, **13**, 398 (1919).



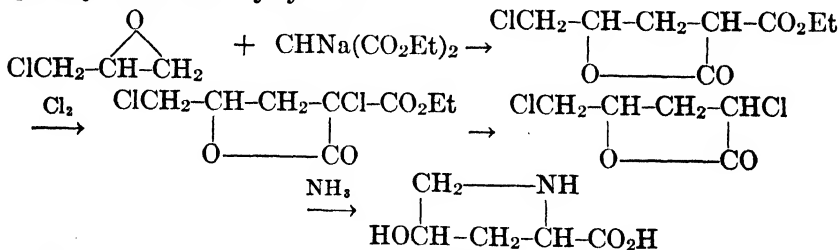
The second, and more convenient, synthesis²⁰³ involves the catalytic reduction of ethyl isonitrosoacetonedicarboxylate:



The synthetic product consists of a mixture of two racemic diastereoisomers, the separation of which has not been accomplished.

Hydroxyproline. γ -Hydroxyproline, which has been stated²⁰⁴ to occur in combination in proteins of animal, but rarely in those of vegetable origin, resembles proline in its solubility in alcohols. Its copper salt, soluble in methyl alcohol, differs from that of proline by being insoluble in absolute ethyl alcohol.¹⁶⁵ Like proline, hydroxyproline is precipitable by Reinecke salt; on the other hand, the rhodanilate is soluble.

Its relation to proline was ascertained by Fischer, at the time of its discovery,²⁰⁵ by its conversion into proline on heating with phosphorus and hydriodic acid. The position of the hydroxyl group was subsequently established by synthesis.²⁰⁶



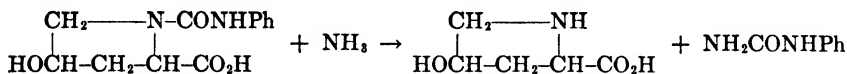
²⁰³ Harington and Randall, *ibid.*, **25**, 1917 (1931).

²⁰⁴ Spörer and Kapfhammer, *Z. physiol. Chem.*, **187**, 84 (1930).

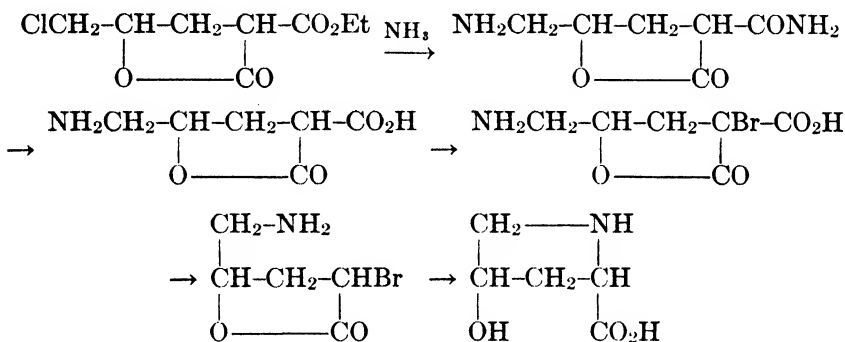
²⁰⁵ Fischer, *Ber.*, **35**, 2660 (1902).

²⁰⁶ Leuchs and collaborators, *Ber.*, **38**, 1937 (1905); **41**, 1726 (1908); **45**, 1960 (1912); **46**, 986 (1913).

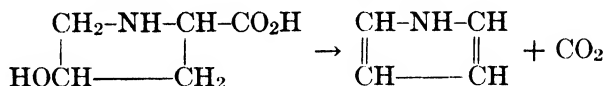
The resulting inactive mixture of diastereoisomers was separated, by crystallization of the copper salts, into two racemic compounds. One of these was converted into the phenylhydantoic acid; the quinine salt of this on fractional crystallization yielded a product identical with the corresponding derivative of the natural amino acid. The synthetic product was converted into the natural hydroxyproline by heating with ammonia.



In a similar synthesis, subsequently performed,²⁰⁷ the order of some of the steps was altered:



On treatment with sodium hypochlorite, hydroxyproline yields a volatile product which gives color reactions with dimethylaminobenzaldehyde and with isatin.²⁰⁸ This is pyrrole.²⁰⁹



On putrefaction, hydroxyproline, like proline, yields δ -aminovaleric acid.²¹⁰

Tyrosine. This phenolic amino acid is very widely distributed as a protein component; however, it is not present in gelatin. Its presence, in combination, is mainly responsible for the yellow color developed by proteins with nitric acid followed by ammonia or alkali (xanthoproteic reaction), and the pink color produced by Millon's reagent (a solution of mercurous nitrate in nitric acid). The latter test is characteristic for

²⁰⁷ Traube, Johow and Tepohl, *Ber.*, **56**, 1861 (1923).

²⁰⁸ Lang, *Z. physiol. Chem.*, **219**, 148 (1933).

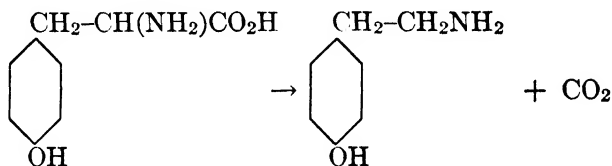
²⁰⁹ Waldschmidt-Leitz and Akabori, *ibid.*, **224**, 187 (1934).

²¹⁰ Keil and Günther, *ibid.*, **221**, 10 (1933).

phenols containing a free *ortho*-position. Tyrosine also yields a red color on treatment in alkaline solution with diazobenzenesulfonic acid²¹¹ and green or red colors with sulfuric acid solutions of formaldehyde or acetaldehyde respectively.^{212, 213} Quantitative color tests, applicable to the estimation of small quantities of tyrosine, depend upon the formation of a blue color with alkaline phosphomolybdate solution²¹⁴ and upon a standardized application of Millon's test.²¹⁵

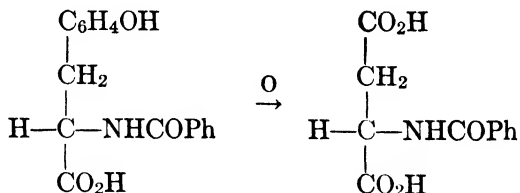
The synthesis of *dl*-tyrosine has been accomplished by the hippuric acid method²¹⁶ and by the introduction of hydroxyl into phenylalanine²¹⁷ by successive nitration, reduction, and diazotization. This second method is of interest as exemplifying the possibility of diazotizing an amino group attached to an aromatic nucleus without affecting another present in a side chain.

When heated under reduced pressure, tyrosine loses carbon dioxide with formation of tyramine.



This pyrolysis proceeds more satisfactorily in a mixture of diphenylmethane and diphenylamine at 260–265° under atmospheric pressure.²⁹

The configurational relationship of natural levorotatory tyrosine to other amino acids has been confirmed by oxidation of the *N*-benzoyl derivative, which yields the benzoyl derivative of natural *l*-aspartic acid.²¹⁸



When tyrosine is subjected to the action of air in presence of the enzyme tyrosinase, which exists in a variety of animal and vegetable

²¹¹ Pauly, *ibid.*, **42**, 508 (1904).

²¹² Denigès, *Compt. rend.*, **130**, 583 (1900); *Bull. soc. chim.*, [4] **3**, 786 (1908).

²¹³ Mörner, *Z. physiol. Chem.*, **37**, 86 (1902).

²¹⁴ Folin and Marenzi, *J. Biol. Chem.*, **83**, 89 (1929).

²¹⁵ Folin and Ciocalteu, *ibid.*, **73**, 627 (1927).

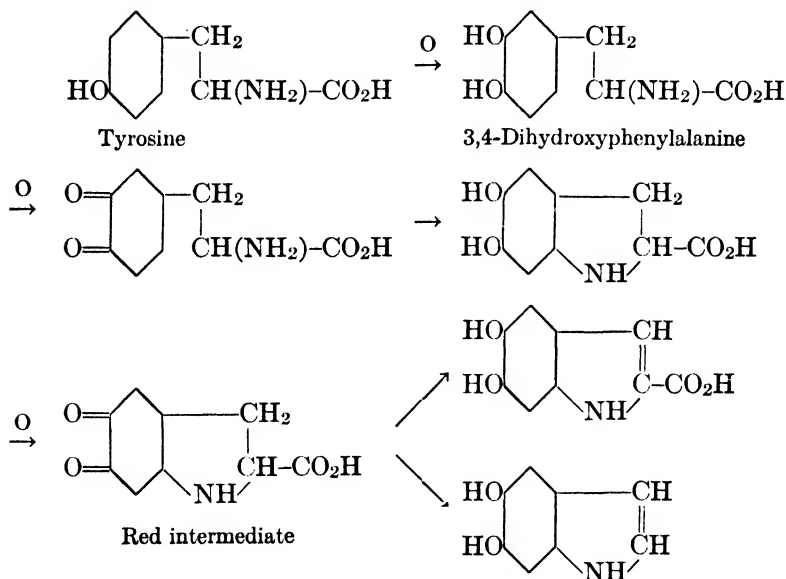
²¹⁶ Erlenmeyer and Halsey, *Ann.*, **307**, 138 (1899).

²¹⁷ Erlenmeyer and Lipp, *Ann.*, **219**, 161 (1883).

²¹⁸ Goldschmidt and Freyss, *Ber.*, **66**, 784 (1933).

tissues (e.g., mealworms or potatoes), oxidation occurs, a dark amorphous, weakly acidic pigment called melanin being formed as the final product. Of the series of reactions which takes place, the first is the introduction of a hydroxyl group in the position *ortho* to that in the tyrosine.²¹⁹ The product, *l*-3,4-dihydroxyphenylalanine, has been isolated from extracts of various vegetable and animal tissues, but has never been obtained from protein hydrolysates. The racemic variety, synthesized by standard processes, has been resolved into its optically active components by crystallizing the brucine salts of the acetyl derivative prepared by hydrogenating the condensation product of protocatechualdehyde and acetylglycine.²²⁰

Being an *o*-dihydroxylic phenol, dihydroxyphenylalanine is readily autoxidizable in alkaline solution, or in neutral solution in the presence of a specific oxidase, with formation of melanin. Apparently the only reaction specifically induced by tyrosinase is the introduction of the hydroxyl group; this reaction, however, also takes place with other phenols. Raper^{219, 221} has shown that the production of melanin involves the intermediate formation of derivatives of indole.



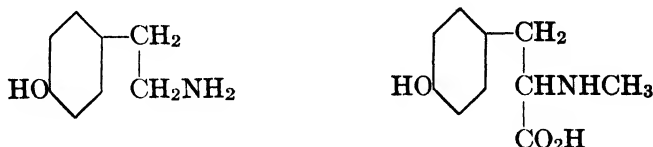
The chemical nature of melanin is still obscure; it appears to represent a further stage in the oxidation of 5,6-dihydroxyindole. Melanin

²¹⁹ Raper, *Biochem. J.*, **20**, 735 (1926).

²²⁰ Harington and Randall, *ibid.*, **25**, 1028 (1931).

²²¹ Raper and collaborators, *ibid.*, **21**, 89, 1370 (1927); **29**, 76 (1935).

or similar products are formed, though more slowly, by the action of tyrosinase upon substances such as tyramine and N-methyltyrosine, closely allied to tyrosine:



Tyrosol (β -*p*-hydroxyphenylethanol) yields a red product, presumably an *o*-quinone.

Like other phenols, tyrosine responds readily to substitution, yielding with chlorine or bromine the corresponding dihalogenated tyrosine. Dibromotyrosine gives no color with Millon's reagent, diazo compounds, or formaldehyde in sulfuric acid, but responds to the phosphomolybdate, ninhydrin (p. 879), and xanthoproteic tests. Its isolation from the products of alkaline hydrolysis of a Norwegian coral has been reported.²²²

3,5-Diiodotyrosine (iodogorgonic acid), which can be synthesized by the action of iodine upon tyrosine in weakly alkaline solution,²²³ is also present, in combined form, in proteins which have been artificially iodinated²²⁴ and in certain natural proteins. It was first isolated²²⁵ from the axial skeleton of a coral (*Gorgonia cavolini*), later from sponge^{226, 227} and from thyroglobulin, the iodine-containing protein of the thyroid gland.^{228, 229}

Diiodotyrosine breaks down, with loss of iodine, in boiling acid solution, but is stable to alkali; hydrolysis of iodine-containing proteins by barium hydroxide leads to racemization, and the product, though it closely resembles that obtained by iodination of *l*-tyrosine, actually is identical with the racemic variety.²³⁰ The natural isomer, however, has been obtained²³¹ by enzymatic hydrolysis of thyroglobulin.

Thyroxine. This iodine-containing amino acid, which exerts the stimulating effect on metabolism characteristic of thyroglobulin, was first isolated by Kendall²³² from an alkali hydrolysate of thyroid substance.

²²² Mörner, *Z. physiol. Chem.*, **88**, 140 (1913).

²²³ Wheeler and Jamieson, *Am. Chem. J.*, **33**, 365 (1905).

²²⁴ Oswald, *Z. physiol. Chem.*, **70**, 310 (1910); **71**, 200; **74**, 290 (1911).

²²⁵ Drechsel, *Z. Biol.*, **33**, 85 (1896).

²²⁶ Wheeler and Mendel, *J. Biol. Chem.*, **7**, 1 (1909).

²²⁷ Oswald, *Z. physiol. Chem.*, **75**, 353 (1911).

²²⁸ Harington and Randall, *Biochem. J.*, **23**, 373 (1929).

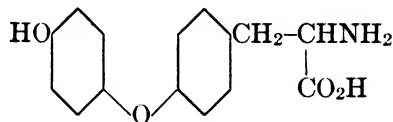
²²⁹ Foster, *J. Biol. Chem.*, **83**, 345 (1929).

²³⁰ Henze, *Z. physiol. Chem.*, **51**, 64 (1907).

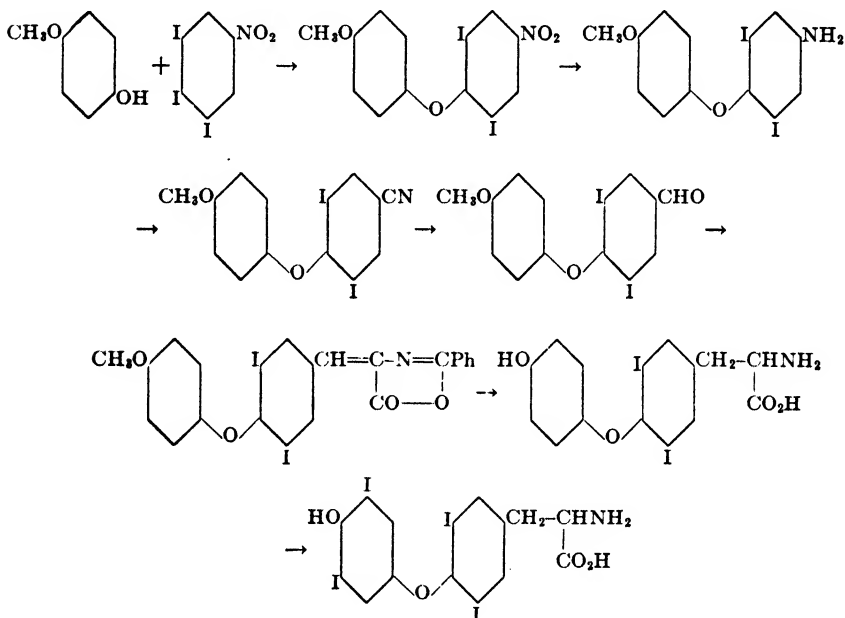
²³¹ Harington and Randall, *Biochem. J.*, **25**, 1032 (1931).

²³² Kendall, *J. Am. Med. Assoc.*, **64**, 2042 (1915); *J. Biol. Chem.*, **39**, 125 (1919).

Its composition and constitution were determined by Harington,²³³ who first established the identity of thyronine (the iodine-free product obtained by catalytic reduction) with the synthetic *p*-hydroxyphenyl ether of tyrosine,



and subsequently, with Barger,²³⁴ synthesized thyroxine itself.



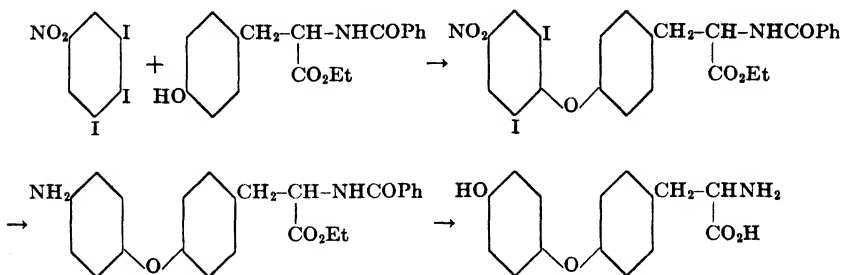
The racemic thyroxine so prepared was identical with that obtained from thyroid, for the instability of thyroxine toward boiling acids rendered necessary the use of barium hydroxide as hydrolysant, with consequent racemization. The optically active isomers were prepared by resolution of the formyl derivative of 3,5-diiodothyronine (the product of the penultimate step in the above synthesis) followed by hydrolysis and iodination. Of these, the levorotatory variety has the same configuration as natural tyrosine; this relationship was established²³⁵ by

²³³ Harington, *Biochem. J.*, **20**, 293, 300 (1926).

²³⁴ Harington and Barger, *ibid.*, **21**, 169 (1927).

²³⁵ Harington and collaborators, *ibid.*, **22**, 1429 (1928); **28**, 68 (1934).

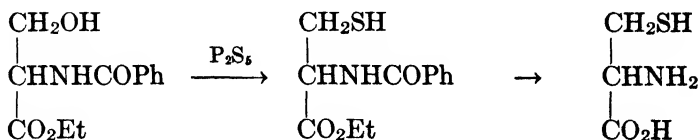
comparison of the corresponding thyronine with a sample synthesized from *l*-tyrosine.



Enzymatic hydrolysis of thyroglobulin leads to *l*-thyroxine²³⁶ which is twice as active physiologically as the racemic product.²³⁷ The same relation holds between the respective potencies of thyroxine in naturally combined form (thyroid protein) and *dl*-thyroxine, from which it appears probable that the physiological activity of the latter is due almost entirely to the *levo* component. The observation of some activity in *d*-thyroxine may conceivably be attributable to its biological conversion to *l*-thyroxine through the corresponding α -keto acid, which has been shown²³⁸ to exert, though to a lower degree, the specific physiological action of thyroxine.

Cysteine and Cystine. These sulfur-containing amino acids, structurally closely related to serine, occur almost universally in proteins; in gelatin, however, they are present only in traces. Although they are readily interconvertible, it has been found possible,⁵ by the application of principles discussed below, to demonstrate the extent to which the one or the other is present in a given protein or its hydrolysate. Cystine is found as such in the urine of persons subject to the obscure metabolic disturbance known as cystinuria, and it forms the major constituent of the urinary calculi often associated with this condition.

Cysteine has been synthesized by the action of phosphorus pentasulfide on benzoylserine ester, followed by hydrolysis;¹⁸¹

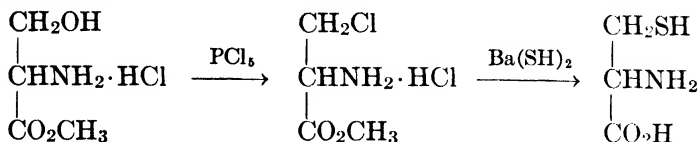


²³⁶ Harington and Salter, *ibid.*, **24**, 456 (1930).

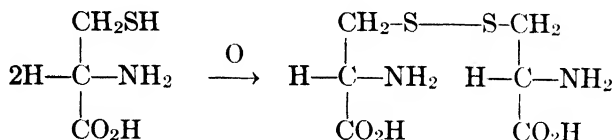
²³⁷ Foster, Palmer and Leland, *J. Biol. Chem.*, **115**, 467 (1936).

²³⁸ Canzanelli, Guild and Harington, *Biochem. J.*, **29**, 1617 (1935).

and from serine ester hydrochloride by successive treatment with phosphorus pentachloride and barium hydrosulfide.²³⁹



The cysteine so produced from natural serine yields on gentle oxidation the natural (levorotatory) variety of cystine.

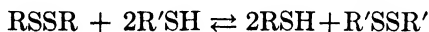


The interconversion of cystine and cysteine is a thermodynamically reversible process, the oxidation-reduction potential of which is apparently characteristic of the general system

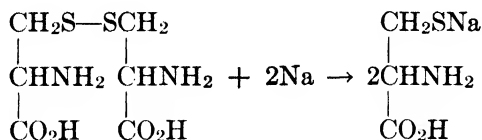


and independent of the nature of the group R.²⁴⁰

Reduction of cystine to cysteine is readily effected, in acid solutions, by means of tin or zinc; in neutral or alkaline solutions an excess of another sulfhydryl compound, such as thioglycollic acid, may be employed for the reduction of the disulfide linkage in proteins.^{241, 242}



For preparative purposes, the action of metallic sodium in liquid ammonia is often convenient.²⁴³



Oxidation of cysteine to cystine may be effected by oxygen in faintly alkaline solution, a reaction catalyzed by traces of salts of iron and other metals which form autoxidizable complexes with cysteine.²⁴⁴ It can

²³⁹ Fischer and Raske, *Ber.*, **41**, 893 (1908).

²⁴⁰ Fruton and Clarke, *J. Biol. Chem.*, **106**, 667 (1934).

²⁴¹ du Vigneaud and collaborators, *ibid.*, **94**, 233 (1931).

²⁴² Goddard and Michaelis, *ibid.*, **112**, 361 (1935).

²⁴³ du Vigneaud, Audrieth and Loring, *J. Am. Chem. Soc.*, **52**, 4500 (1930).

²⁴⁴ Michaelis and Schubert, *ibid.*, **52**, 4418 (1930); **53**, 3851 (1931); Schubert, *ibid.*, **54**, 4077 (1932).

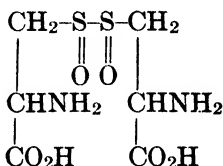
also be carried out in acid solution by means of iodine; this reaction forms the basis of a method for the quantitative estimation of cysteine.²⁴⁵

With bromine water, the sulfur atoms in cysteine (and cystine) are rapidly oxidized to sulfonic acid groups, with production of cysteic acid.



The same reaction takes place, relatively slowly, with iodine.²⁴⁶

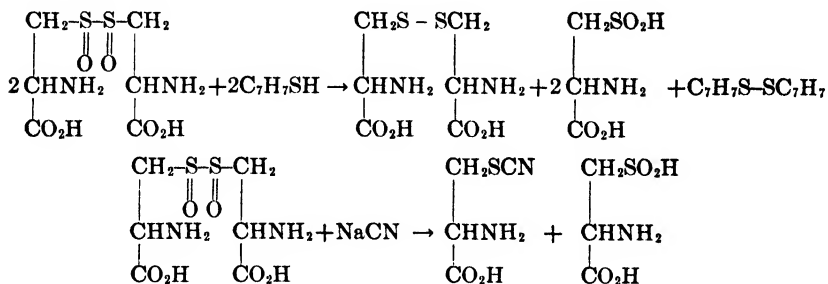
Intermediate oxidation products have been isolated. By the action of perbenzoic acid in acetonitrile, cystine perchlorate is converted into a disulfoxide,



which is reduced to cystine by hydriodic acid and oxidized to cysteic acid by excess of iodine. In acid solution, spontaneous dismutation of the disulfoxide occurs²⁴⁷ with formation of cystine and a sulfinic acid.



The latter is also formed by the action of hydrogen peroxide upon the complex potassium cobalto biscysteinate.²⁴⁸ The dismutation of the disulfoxide, which is paralleled by the actions of *p*-thiocresol and sodium cyanide,



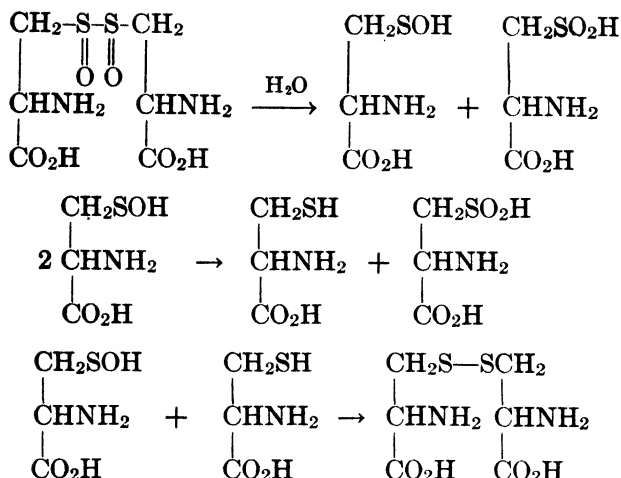
possibly involves a hypothetical, highly reactive and unstable sulfenic acid.

²⁴⁵ Okuda, *J. Biochem. (Japan)*, **5**, 207 (1925); *Proc. Imp. Acad. (Tokyo)* **5**, 246 (1929).

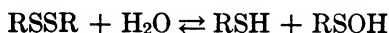
²⁴⁶ Friedmann, *Beitr. chem. Physiol. Path.*, **3**, 27 (1903); Yamazaki, *J. Biochem. (Japan)*, **12**, 207 (1930).

²⁴⁷ Toennies and Lavine, *J. Biol. Chem.*, **113**, 571, 583 (1936).

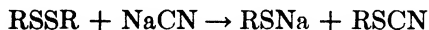
²⁴⁸ Schubert, *J. Am. Chem. Soc.*, **55**, 3336 (1933).



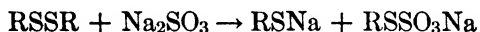
The reaction between cysteine and the hypothetical sulfenic acid appears to form one aspect of an equilibrium reaction undergone by cystine and other disulfides, especially in alkaline solution.²⁴⁹



This equilibrium²⁵⁰ serves to explain the behavior of cystine toward cyanide,^{251, 252}



and sulfite.²⁵³



In this connection mention may be made of the interesting observation²⁵⁴ that cystine, cysteine, and thioglycollic acid, when given in sufficiently large doses, can act as antidotes against cyanide poisoning.

Like other thiol compounds, cysteine forms water-insoluble derivatives with silver, mercury, and cuprous copper. Such precipitates are also produced from cystine; with silver and mercuric ions, reduction of the disulfide linkage takes place at the expense of a portion (about one-sixth) which is not precipitated but appears as cysteic acid;^{255, 256} with

²⁴⁹ Schöberl and collaborators, *Ann.*, **507**, 111 (1933); *Ber.*, **67**, 1545 (1934); *Naturwissenschaften*, **24**, 391 (1935).

²⁵⁰ Shinohara and Kilpatrick, *J. Biol. Chem.*, **105**, 241 (1934).

²⁵¹ Mauthner, *Z. physiol. Chem.*, **78**, 28 (1912).

²⁵² Pulewka and Winzer, *Arch. expil. Path. Pharmacol.*, **138**, 154 (1928).

²⁵³ Clarke, *J. Biol. Chem.*, **97**, 235 (1932).

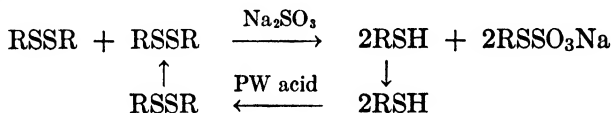
²⁵⁴ Voegtlin, Johnson, and Dyer, *J. Pharmacol.*, **27**, 467 (1926).

²⁵⁵ Vickery and Leavenworth, *J. Biol. Chem.*, **86**, 129 (1930).

²⁵⁶ Simonsen, *ibid.*, **94**, 323 (1931).

excess of cuprous chloride, on the other hand, a quantitative yield is obtainable as the disulfide linkage is reduced by the cuprous ion.²⁵⁷

In colorimetric methods for the analytical estimation of cystine the first step consists in the rupture of the disulfide linkage to produce cysteine. The procedures elaborated by Folin,²¹⁴ and the modifications of these,^{258, 259} depend upon the blue color produced by the action of cysteine (and other sulfhydryl compounds) upon phosphotungstic acid. Lugg²⁶⁰ has shown that the intensity of this color from pure cysteine alone is the same as that from a corresponding quantity of cystine in presence of sodium sulfite in excess; cysteine with sulfite yields twice the intensity. From this it is clear that, by the oxidizing action of the phosphotungstic reagent in the presence of sulfite, all the cystine (or cysteine) is ultimately converted into the non-reducing thiosulfonic derivative:



The development of sulfhydryl compounds of a characteristic purple color with nitroprusside has been applied to the estimation of cystine.²⁶¹ In this case the disulfide linkage is opened by double decomposition with cyanide.

More specific is the process of Sullivan.²⁶² In this, cystine is treated successively with cyanide and 1,2-naphthoquinone-4-sulfonic acid; the color developed by cysteine is not, in contrast to that from other amino acids, discharged on addition of sodium hydrosulfite. As in the Folin process, cysteine yields twice the color intensity furnished by cystine.²⁶³ Difficulty in obtaining reproducible results by this procedure is ascribable to its complicated and highly empirical character; the presence of adventitious substances and slight variations in technique affect the color intensity. Under certain conditions exposure of the final solution to air leads to increase in color;²⁶⁴ it seems possible that the specific colored product may be present to a variable degree in the form of an autoxidizable leuco derivative. A color reaction,

²⁵⁷ Rossouw and Wilken-Jorden, *Biochem. J.*, **29**, 219 (1935).

²⁵⁸ Tompsett, *ibid.*, **25**, 2014 (1931).

²⁵⁹ Shinohara, *J. Biol. Chem.*, **109**, 665 (1935).

²⁶⁰ Lugg, *Biochem. J.*, **26**, 2144 (1932).

²⁶¹ Brand, Harris, and Biloan, *J. Biol. Chem.*, **86**, 315 (1930).

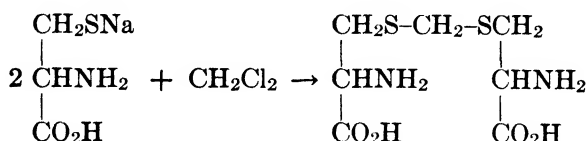
²⁶² Sullivan, *U. S. Pub. Health Repts.*, **41**, 1030 (1926); **44**, 1421 (1929); Sullivan and Hess, *ibid.*, **44**, 1599 (1929).

²⁶³ Lugg, *Biochem. J.*, **27**, 668 (1933).

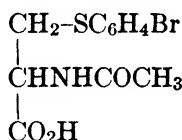
²⁶⁴ Bushill, Lampitt, and Baker, *ibid.*, **28**, 1293 (1934).

apparently specific and possibly analogous to the preceding, is given by cysteine with *o*-benzoquinone.²⁶⁵

Derivatives of cysteine in which the sulfhydryl hydrogen atom is replaced by alkyl groups are of course devoid of actual or potential reducing properties and do not respond to the specific color tests. They may conveniently be synthesized by the action of alkyl halides upon the sodium derivative of cysteine in aqueous²⁶⁶ or liquid ammonia²⁶⁷ solution. An interesting member of this series is djenkolic acid, present in the Djenkol bean,²⁶⁸ the synthesis of which from *l*-cysteine has been accomplished by the liquid ammonia technique.²⁶⁹



S-Aryl derivatives of cysteine have been prepared by the action of diazo compounds upon cysteine;^{270,266} on acetylation they are converted into mercapturic acids. Bromobenzene, given by mouth to a dog, is excreted in the urine as *p*-bromophenylmercapturic acid,^{271, 272}



in unstable combination with some unknown compound, possibly a glycuronic acid. The extent to which the bromobenzene is converted to the mercapturic acid depends largely upon the amount of cystine available in the body.^{273, 274}

Cysteine reacts with aqueous formaldehyde to yield an optically active thiazolidinecarboxylic acid²⁷⁵

²⁶⁵ Dyer and Baudisch, *J. Biol. Chem.*, **95**, 483 (1932).

²⁶⁶ Clarke and Inouye, *ibid.*, **94**, 541 (1931).

²⁶⁷ du Vigneaud, Loring, and Craft, *ibid.*, **105**, 481 (1934).

²⁶⁸ van Veen and Hyman, *Rec. trav. chim.*, **54**, 493 (1935).

²⁶⁹ du Vigneaud and Patterson, *J. Biol. Chem.*, **114**, 533 (1936).

²⁷⁰ Friedmann, *Beitr. chem. Physiol. Path.*, **4**, 486 (1904).

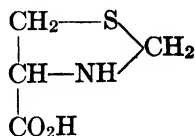
²⁷¹ Baumann and Preusse, *Ber.*, **12**, 806 (1879); *Z. physiol. Chem.*, **5**, 309 (1881).

²⁷² Jaffé, *Ber.*, **12**, 1092 (1879).

²⁷³ Kapfhammer, *Z. physiol. Chem.*, **116**, 302 (1921).

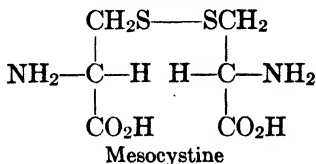
²⁷⁴ Muldoon, Shiple, and Sherwin, *J. Biol. Chem.*, **59**, 675 (1924).

²⁷⁵ Ratner and Clarke, *J. Am. Chem. Soc.*, **59**, 200 (1937).

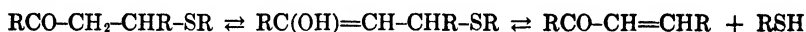


in which the basic properties are markedly weaker than in cysteine.

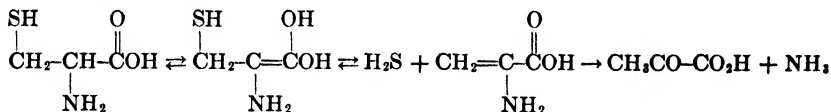
The stereochemical relations of cystine are of interest. On prolonged boiling with hydrochloric acid,²⁷⁶ *l*-cystine loses its optical activity, being converted into a mixture of the truly racemic and the *meso* varieties.²⁷⁷ The latter is incapable of resolution:



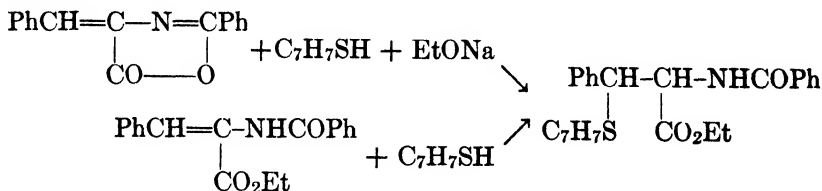
Like serine, cysteine and cystine break down in hot alkaline solution to yield ammonia and pyruvic acid; sulfide is also formed. The decomposition is accelerated by the presence of lead oxide, pyruvate, or aromatic aldehydes; acylation also increases the instability towards alkali. Nicolet, from a consideration of the behavior of β -ketonic sulfides toward alkali,



has suggested²⁷⁸ that the alkaline decomposition of cysteine takes the following course:



supporting this view by the synthesis of cysteine derivatives from mercaptans and unsaturated azlactones or the corresponding open-chain esters.

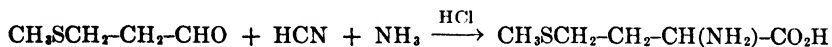


²⁷⁶ Hoffmann and Gortner, *ibid.*, **44**, 341 (1922).

²⁷⁷ du Vigneaud and collaborators, *J. Biol. Chem.*, **94**, 243 (1931); **98**, 577 (1932); **102**, 287 (1933); **107**, 267 (1934).

²⁷⁸ Nicolet, *J. Am. Chem. Soc.*, **53**, 3066 (1931); **54**, 1998 (1932); *J. Biol. Chem.*, **95**, 389 (1932).

Methionine. Although it had for many years been evident that sulfur must exist in proteins in a form other than that of cystine or cysteine, the first definite hint of its nature was afforded in 1914 by the observation of Mörner²⁷⁹ that oxidation of proteins with nitric acid leads to the formation of methanesulfonic acid in yields totally unrelated to their content of cystine. That this compound is not formed from pure cystine was shown by direct experiment. In 1923 Mueller²⁸⁰ isolated from protein hydrolysates a crystalline amino acid isomeric with ethylcystine but differing from it in being stable to boiling alkali. The same product was shortly thereafter obtained from yeast by extraction with 80 per cent alcohol. Its constitution was established by Barger and Coyne,²⁸¹ who synthesized the racemic form by applying the Strecker synthesis to β -methylthiolpropionaldehyde.



More advantageous syntheses, later developed, involve the application of the malonic ester²⁸² and the phthaliminomalonic ester²⁸³ procedures to β -chloroethyl methyl sulfide.

L-Methionine is now recognized as an almost universal constituent of proteins, from which it is liberated by acid hydrolysis and by the action of proteolytic enzymes.²⁸⁴ Like other monoamino monocarboxylic acids, it is extractable from neutral solution by butyl alcohol; it is a frequent contaminant of leucine of protein origin,²⁸⁵ from which it can be separated as a sparingly soluble mercury derivative.²⁸⁶

Normally it is metabolized to sulfate to about the same extent as cystine; in the cystinuric, it is excreted largely as cystine.²⁸⁷ It favors the production of *p*-bromophenylmercapturic acid (p. 917) in dogs receiving bromobenzene,²⁸⁸ and stimulates the growth of rats on diets from which cystine is absent.^{289, 290}

It thus appears that the body can convert methionine into cystine. The manner in which this change is brought about is not clear. It has

²⁷⁹ Mörner, *Z. physiol. Chem.*, **93**, 175 (1914).

²⁸⁰ Mueller, *J. Biol. Chem.*, **56**, 156 (1923).

²⁸¹ Barger and Coyne, *Biochem. J.*, **22**, 1417 (1928).

²⁸² Windus and Marvel, *J. Am. Chem. Soc.*, **52**, 2575 (1930).

²⁸³ Barger and Weichselbaum, *Biochem. J.*, **25**, 997 (1931).

²⁸⁴ Pirie, *ibid.*, **26**, 2041 (1932); **27**, 202 (1933).

²⁸⁵ Mueller, *Science*, **81**, 50 (1935).

²⁸⁶ Hill and Robson, *Biochem. J.*, **28**, 1008 (1934).

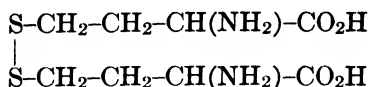
²⁸⁷ Brand, Cahill, and Harris, *J. Biol. Chem.*, **109**, 69 (1935).

²⁸⁸ White and Lewis, *ibid.*, **98**, 607 (1932).

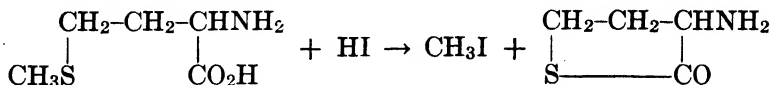
²⁸⁹ Jackson and Block, *ibid.*, **98**, 465 (1932).

²⁹⁰ du Vigneaud, Dyer, and Harmon, *ibid.*, **101**, 719 (1933).

been suggested²⁹¹ that the primary reaction is the demethylation to homocysteine, a conversion which can be effected in the laboratory by drastic procedures only. On heating in 60 per cent sulfuric acid methionine is converted into homocysteine



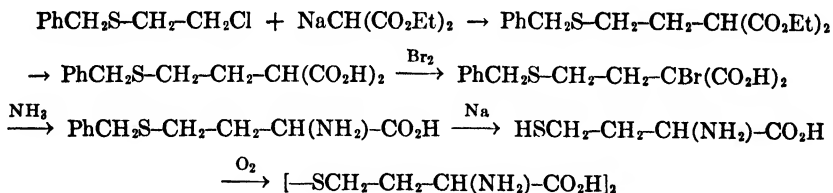
with only slight racemization,²⁹² and in boiling hydriodic acid it breaks down into methyl iodide and the thiolactone of homocysteine,



a reaction which forms the basis for an analytical method for the estimation of methionine.²⁹³

l(-)-Methionine is converted by nitrous acid and by the action of *B. subtilis* into *l*(-)- α -hydroxy- γ -methiobutyric acid; on the other hand, it is converted by *Oidium lactis* into the *d*(+)-hydroxy acid.²⁹⁴ Both isomers can be utilized for growth in lieu of cystine or methionine, which is not true of the α -hydroxy acid corresponding to cystine.

Homocysteine has been synthesized²⁹⁵ by the malonic ester method. During the early stages of the synthesis, the sulfur atom is protected by a benzyl group, which is later removed (as bibenzyl and toluene) by the action of sodium in liquid ammonia.



The homocysteine, which in the last step is oxidized to homocystine, is stable only in alkaline solution; in presence of acid it loses water to form the thiolactone,²⁹⁶ which has no reducing action on iodine until the ring has been opened by alkali. In hot 60 per cent sulfuric acid

²⁹¹ Virtue and Lewis, *ibid.*, **104**, 59 (1934).

²⁹² du Vigneaud and collaborators, *ibid.*, **99**, 135 (1932); **109**, 97 (1935).

²⁹³ Baernstein, *ibid.*, **106**, 451 (1934); **115**, 24 (1936).

²⁹⁴ Akobe, *Z. physiol. Chem.*, **244**, 14 (1936).

²⁹⁵ Patterson and du Vigneaud, *J. Biol. Chem.*, **111**, 393 (1935).

²⁹⁶ Riegel and du Vigneaud, *ibid.*, **112**, 149 (1935).

homocysteine undergoes oxidation to homocystine to a greater extent than ring closure—a finding which partially explains the formation of homocystine from methionine. Cold or more dilute sulfuric acid favors thiolactonization.

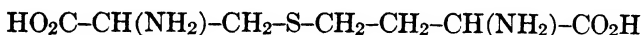
Homocystine can be converted into methionine by successively treating it in liquid ammonia solution with sodium and methyl iodide.²⁹⁰ By resolving S-benzylhomocysteine into its optical components and subjecting the products to the reactions outlined above, it has been possible to prepare the active isomers of homocystine and methionine.²⁹⁷

The higher homologs, homomethionine and pentocystine,



have been prepared by analogous methods. Unlike the preceding members of the series, neither is utilizable for growth in place of cystine.²⁹⁸

An amino acid to which the constitution



is ascribed has been isolated from the products of the action of sodium sulfide on wool.²⁹⁹ Since the existence of this compound, which contains the structures of both cysteine and methionine, has not been confirmed, doubt exists as to whether it is a true protein component or merely an artifact.

Lysine. The classical methods devised by Kossel and Kutscher,³⁰⁰ for the separation of the three basic amino acids or “hexone bases,” form the basis for the modern procedures developed by Vickery³⁰¹ and Block.³⁰² An excess of silver sulfate or nitrate is added to a sulfuric acid hydrolysate previously adjusted to pH 3–6 with barium hydroxide, and the acidity is further reduced by gradual addition of more barium hydroxide. At pH 7.0–7.4 histidine, at pH 13–14 arginine precipitate, as their silver derivatives. Lysine, which remains in the filtrate, is subsequently thrown down as its phosphotungstate and finally converted into the picrate.

Although lysine is extremely soluble in water, it tends to separate

²⁹⁷ du Vigneaud and Patterson, *ibid.*, **109**, 97 (1935).

²⁹⁸ du Vigneaud and collaborators, *ibid.*, **106**, 401 (1934); **108**, 73 (1935).

²⁹⁹ Küster and Irion, *Z. physiol. Chem.*, **184**, 225 (1929).

³⁰⁰ Kossel and Kutscher, *ibid.*, **31**, 165 (1900).

³⁰¹ Vickery and collaborators, *J. Biol. Chem.*, **75**, 115 (1927); **86**, 107 (1930); **93**, 105 (1931).

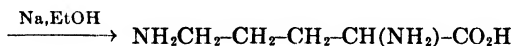
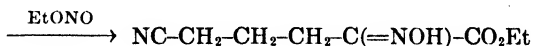
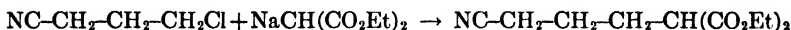
³⁰² Block, *ibid.*, **106**, 457 (1934).

from a neutralized casein hydrolysate in association with the sparingly soluble tyrosine, with which it appears to form a double salt.³⁰³

Lysine is a fairly general constituent of proteins, though it is absent, or nearly so, from alcohol-soluble proteins of vegetable origin. It occurs as such, together with other amino acids, in germinating seeds, in which proteins are being actively synthesized. Although it is not a constituent of normal urine, it has been isolated from that of cystinurics³⁰⁴ either as such or as the ureidohydantoin formed by the action of urea.³⁰⁵

In only those proteins which contain lysine can the amino group be detected.³⁰⁶ The lysine appears to be linked with other amino acids through its carboxyl and α -amino groups, the terminal group supplying practically all the free amino groups ascertainable in proteins by means of nitrous acid,³⁰⁷ for the values so obtained correspond closely to one-half of those for lysine nitrogen. Moreover, no lysine is obtainable from proteins which have been deaminized by nitrous acid.^{308, 309} In the reaction with nitrous acid (Van Slyke procedure) evolution of nitrogen from proteins can be almost completely suppressed by chilling to 0–3°; at this temperature lysine yields only one-half of its nitrogen, but at 32° yields all in 5 minutes. It is the terminal group of lysine which is the less reactive, for alanine responds readily and completely to nitrous acid at 3°.³¹⁰ Proteins which have been benzoylated or benzenesulfonylated yield on hydrolysis the ϵ -benzoyl³¹¹ and ϵ -benzenesulfonyl³¹² derivatives of lysine, respectively.

dl-Lysine has been synthesized by reducing ethyl α -isonitroso- δ -cyanovalerate with sodium and alcohol;³¹³



by reduction of the condensation product of γ -chlorobutyronitrile and

³⁰³ Fischer and Abderhalden, *Z. physiol. Chem.*, **42**, 540 (1904).

³⁰⁴ Ackermann and Kutscher, *Z. Biol.*, **57**, 355 (1911).

³⁰⁵ Hoppe-Seyler, *Z. physiol. Chem.*, **214**, 267 (1933).

³⁰⁶ Kossel and collaborators, *ibid.*, **76**, 457; **81**, 274 (1912).

³⁰⁷ Van Slyke and Birchard, *J. Biol. Chem.*, **16**, 539 (1914).

³⁰⁸ Skraup and Kaas, *Ann.*, **351**, 379 (1906).

³⁰⁹ Kossel and Weiss, *Z. physiol. Chem.*, **78**, 402 (1912).

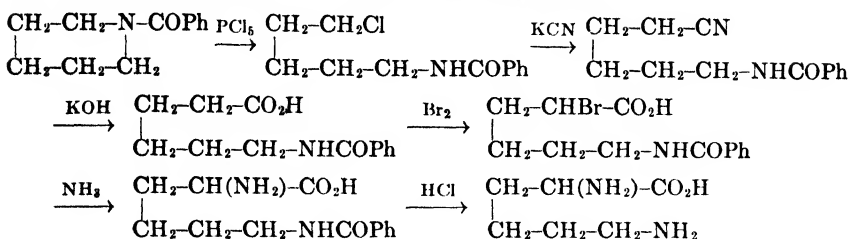
³¹⁰ Sure and Hart, *J. Biol. Chem.*, **31**, 527 (1917).

³¹¹ Goldschmidt and Kinsky, *Z. physiol. Chem.*, **183**, 244 (1929).

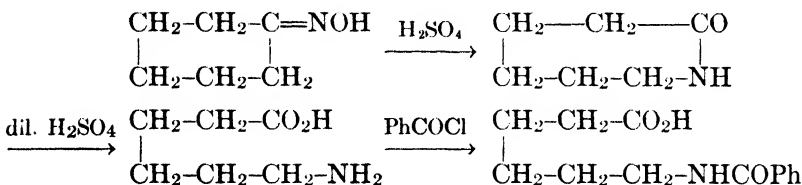
³¹² Gurin and Clarke, *J. Biol. Chem.*, **107**, 395 (1934).

³¹³ Fischer and Weigert, *Ber.*, **35**, 3772 (1902).

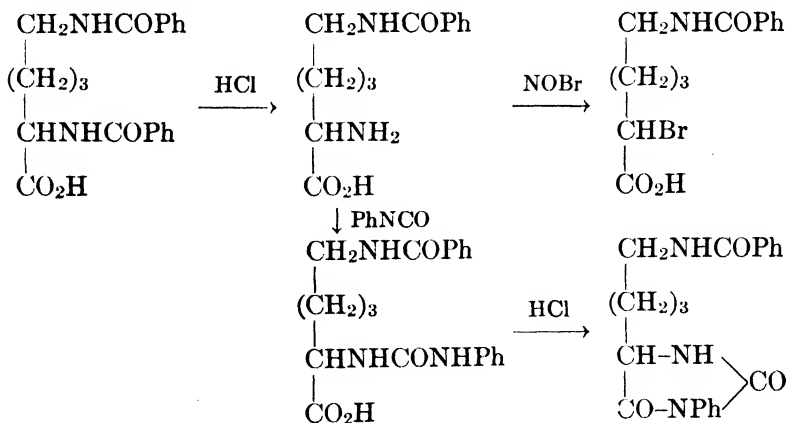
sodium ethyl phthaliminomalonate, followed by hydrolysis;³¹⁴ and from benzoylpiperidine³¹⁵ by the following steps:



In a convenient modification³¹⁶ of this synthesis, the intermediate ϵ -benzoylamino-caproic acid is prepared from cyclohexanoneoxime.



Lysine forms a dibenzoyl derivative, lysuric acid,³¹⁷ which on partial hydrolysis by acid yields a monobenzoyl compound.³¹³ On treatment with nitrosyl bromide this is converted into α -bromo- ϵ -benzoylamino-caproic acid identical with that obtained from benzoylpiperidine, and with phenyl isocyanate it yields a phenylureide readily convertible into a phenylhydantoin.³¹⁸ It must therefore be the ϵ -monobenzoyllysine:



³¹⁴ Sørensen, *Compt. rend. trav. lab. Carlsberg*, **6**, 1 (1903) [*Chem. Zentr.*, (II), 33 (1903)].

³¹⁵ v. Braun, *Ber.*, **42**, 839 (1909).

³¹⁶ Eck and Marvel, *J. Biol. Chem.*, **106**, 387 (1934).

³¹⁷ Drechsel, *Ber.*, **28**, 3189 (1895).

³¹⁸ Karrer and Ehrenstein, *Helv. Chim. Acta*, **9**, 323 (1926).

Benzoylated proteins, on hydrolysis, yield the same monobenzoyl lysine, the constitution of which has been confirmed by oxidation to δ -benzoyl-aminovaleric acid.³¹¹ Alkaline hydrolysis of lysuric acid likewise causes the removal of the α -benzoyl group.³¹⁹ On the other hand, dibenzenesulfonyl lysine on acid hydrolysis loses a benzenesulfonyl group exclusively from the ϵ -position.³¹²

Arginine. The properties of arginine are profoundly influenced by the presence of the strongly basic (p. 891) guanidino group, which is reflected by the contrast between its titration curve and that of lysine³²⁰ in the region of pH above 10. When amino acids are titrated in 85 per cent alcohol or in presence of formalin, amino groups lose their basic function (cf. p. 875); the guanidino group does not.^{19, 321} The observation⁴⁴ that acetylarginine is readily racemized in aqueous solution by acetic anhydride, in the absence of sodium acetate or other catalyst (cf. p. 927), can be explained by the strongly basic character of the guanidino group.

Arginine appears to be a universal component of proteins, from which it is partially split off by enzymes during the early stages of proteolysis.^{322, 323} It is particularly abundant in the protamines of fish sperm; these highly basic polypeptides contain up to 90 per cent of their nitrogen in the form of arginine.^{300, 324, 325}

The guanidino group in proteins is selectively decomposed by sodium hypochlorite or hot alkalis.³²⁶ The products, which yield but little arginine on hydrolysis, are no longer soluble in dilute acids and are not digestible by pepsin, but are readily hydrolyzed by trypsin. When sodium hypochlorite is added to an alkaline solution of arginine with α -naphthol, a red color is produced. This test is positive with proteins containing combined arginine³²⁷ and with monosubstituted guanidines such as methylguanidine or glycocyamine; it is also given by *sym*-dimethylguanidine and *sym*-trimethylguanidine but not by asymmetrically di- and trisubstituted guanidines (e.g., *as*-dimethylguanidine, glycocyamidine, creatine) nor guanidine itself.³²⁸ It has been developed into a quantitative procedure for the estimation of arginine.^{329, 330}

³¹⁹ Karrer and Ehrenstein, *ibid.*, **9**, 1063 (1926).

³²⁰ Schmidt, Kirk and Appleman, *J. Biol. Chem.*, **88**, 285 (1930).

³²¹ Levy, *ibid.*, **109**, 365 (1935).

³²² Dauphinee and Hunter, *Biochem. J.*, **24**, 1128 (1930).

³²³ Lieben and Lieber, *Biochem. Z.*, **275**, 38 (1934).

³²⁴ Kossel and Dakin, *Z. physiol. Chem.*, **41**, 407 (1904).

³²⁵ Taylor, *J. Biol. Chem.*, **5**, 389 (1909).

³²⁶ Sakaguchi, *J. Biochem. (Japan)*, **5**, 143, 159 (1925).

³²⁷ Sakaguchi, *ibid.*, **5**, 25, 133 (1925).

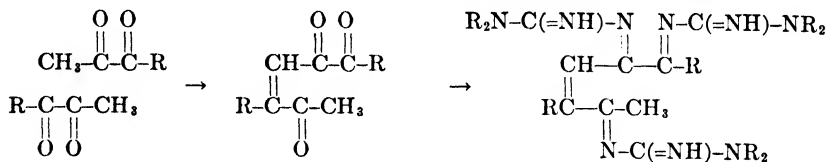
³²⁸ Poller, *Ber.*, **59**, 1927 (1926).

³²⁹ Weber, *J. Biol. Chem.*, **86**, 217; **88**, 353 (1930).⁷

³³⁰ Jorpes and Thorén, *Biochem. J.*, **26**, 1504 (1932).

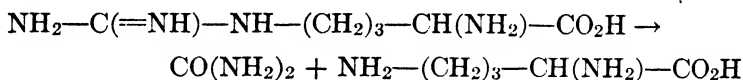
Proteins also give the color, in intensities equivalent to their content of combined arginine.

Arginine, in common with other guanidino derivatives containing two hydrogen atoms attached to a single nitrogen atom, develops a violet color on treatment with biacetyl or acetyl benzoyl in alkaline solution.^{331, 332} This color reaction, which has been placed on a quantitative basis, depends upon the following series of reactions:

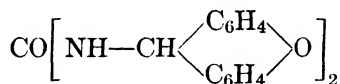


Creatine (p. 891) responds to this test; creatinine does not.

Another, and far more specific, method is based upon the hydrolysis of arginine into ornithine and urea under the influence of an enzyme, arginase, present in mammalian liver.³³³



The urea so formed is estimated either as ammonia after hydrolysis by urease³³⁴ or by treatment with xanthidrol,³³⁵ which causes the quantitative precipitation of dixanthylurea.³³⁶



The conversion of arginine to ornithine and urea can also be effected by boiling with barium hydroxide;³³⁷ on boiling with 20 per cent (or stronger) sodium hydroxide^{338, 339} arginine is converted into ornithine and two equivalents of ammonia.

The method of separating arginine from other amino acids by its precipitation at high alkalinity as a silver derivative analogous to the insoluble compound $\text{CH}_3\text{N}_3\text{Ag}_2 \cdot \text{H}_2\text{O}$ formed by guanidine under similar conditions has been supplemented, for analytical and preparative pur-

³³¹ Harden and Norris, *J. Physiol.*, **42**, 332 (1911).

³³² Lang, *Z. physiol. Chem.*, **208**, 273 (1932).

³³³ Kossel and Dakin, *ibid.*, **41**, 321; **42**, 181 (1904).

³³⁴ Hunter and Dauphinee, *J. Biol. Chem.*, **85**, 627 (1950).

³³⁵ Bonot and Cahn, *Compt. rend.*, **184**, 246 (1927).

³³⁶ Fosse, *ibid.*, **158**, 1076 (1914); *Ann. Chim.*, [9], **6**, 13 (1916).

³³⁷ Schulze and collaborators, *Ber.*, **24**, 2701 (1891); **30**, 2879 (1897).

³³⁸ Van Slyke, *J. Biol. Chem.*, **10**, 15 (1911).

³³⁹ Plimmer, *Biochem. J.*, **10**, 115 (1916).

poses, by a process based on the observation that arginine forms a sparingly soluble salt with 2,4-dinitro-1-naphthol-7-sulfonic (flavianic) acid.^{340, 301} The flavianate may conveniently be converted, by means of strong hydrochloric acid, in which the free flavianic acid is not freely soluble, into the very soluble dihydrochloride; this yields the crystalline monohydrochloride on treatment with aniline.³⁴¹

Arginine also forms with benzaldehyde a water-insoluble derivative which separates selectively from protein hydrolysates rendered strongly alkaline.⁶⁹ Benzylidene arginine is readily decomposed into benzaldehyde and a salt of arginine on warming with dilute mineral acid. The inability of this compound to form a sodium salt is attributable to the failure of the alkali metal to replace the equally strongly basic guanidino group present in the molecule. A similar effect is observed with the hydantoic acid formed by the action of potassium cyanate upon arginine monohydrochloride;³⁴² the solubility of this product in water is not increased by the addition of alkali.

On treatment with sodium nitrite and acetic acid in the Van Slyke procedure, arginine gives rise to only one molecule of nitrogen,^{343, 344, 345} although guanidine reacts rapidly with nitrous acid in the presence of mineral acid, it does not do so in acetic acid,³⁴⁶ and the guanidino group in arginine behaves in the same way. Proteins, on hydrolysis after treatment with sodium nitrite and acetic acid, yield arginine but no lysine.³⁴⁷

The guanidino group is resistant to the action of barium permanganate, by which arginine is oxidized first to γ -guanidinobutyric acid³⁴⁸ and finally to guanidine.³⁴⁹ Proteins yield guanidine on similar treatment.³⁵⁰

Like guanidine, arginine can be nitrated.³⁵¹ Nitroarginine, which can also be obtained by hydrolysis of a nitrated protamine, lacks the strongly basic character of arginine, which is regenerated by catalytic hydrogenation.³⁵²

³⁴⁰ Kossel and Gross, *Z. physiol. Chem.*, **135**, 167 (1924).

³⁴¹ Cox, *J. Biol. Chem.*, **78**, 475 (1928).

³⁴² Boon and Robson, *Biochem. J.*, **29**, 2573 (1935).

³⁴³ Van Slyke, *J. Biol. Chem.*, **9**, 185 (1911).

³⁴⁴ Plimmer, *Biochem. J.*, **18**, 105 (1924).

³⁴⁵ Hunter, *J. Biol. Chem.*, **82**, 731 (1929).

³⁴⁶ Hynd and Macfarlane, *Biochem. J.*, **20**, 1264 (1926).

³⁴⁷ Traxl, *Monatsh.*, **29**, 59 (1908).

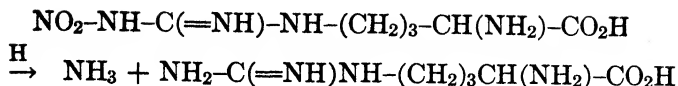
³⁴⁸ Kutscher, *Z. physiol. Chem.*, **32**, 413 (1901).

³⁴⁹ Bénech and Kutscher, *ibid.*, **32**, 278 (1901).

³⁵⁰ Lossen, *Ann.*, **201**, 369 (1880); Otori, *Z. physiol. Chem.*, **43**, 86 (1904); Kutscher and Schenck, *Ber.*, **38**, 455 (1905).

³⁵¹ Kossel and Kennaway, *Z. physiol. Chem.*, **72**, 486 (1911).

³⁵² Bergmann, Zervas, and Rinke, *ibid.*, **224**, 40 (1934).



This reaction has been adapted to meet the needs of peptide synthesis.

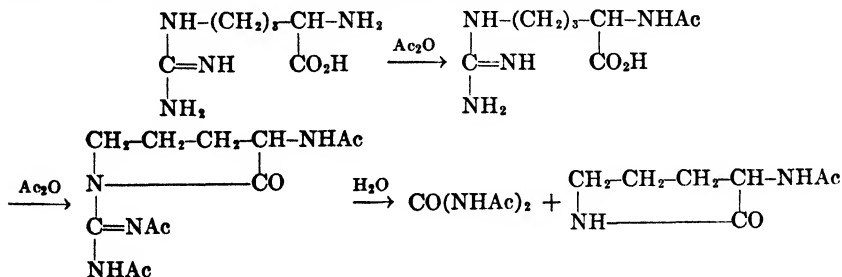
An analogous compound, arginine-phosphoric acid, has been isolated from the muscle of marine invertebrates, in which it plays the part taken by creatine-phosphoric acid (p. 893) in muscular contraction of vertebrates.³⁵³ Arginine-phosphoric acid



in which the dissociation constant of the guanidino group has been lowered by the introduction of the phosphoryl group, is hydrolyzed to arginine and phosphoric acid during muscular activity and is resynthesized during relaxation.

Acyl derivatives of arginine can be prepared by the usual methods, the α -amino group being far more readily acylated than the guanidino group. With benzoyl chloride and alkali, a dibenzoyl derivative is formed;³⁵⁴ on the other hand, in attempts to prepare the corresponding di- β -naphthalenesulfonyl derivative³⁵⁵ only one acyl group could be introduced. On treatment with benzenesulfonylchloride in presence of potassium carbonate, arginine forms the α -monobenzenesulfonyl derivative, but a second benzenesulfonyl group may be introduced by the use of concentrated sodium hydroxide in excess.³⁵⁶

With acetic anhydride in the cold, arginine yields a monoacetyl derivative, which has become racemized under the combined influence of the excess of anhydride and the strongly polar guanidino group (cf. p. 872). Boiling acetic anhydride leads to the production of a triacetyl anhydroarginine,³⁵⁷ which on treatment with water breaks down into diacetylurea and β -acetamino- α -piperidone.



³⁵³ Meyerhof and Lohmann, *Biochem. Z.*, **196**, 22, 49 (1928); Riesser and Hansen, *Z. physiol. Chem.*, **219**, 62 (1933).

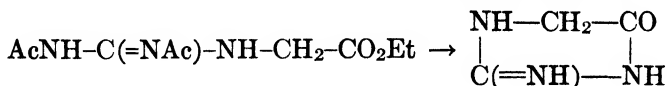
³⁵⁴ Gulewitsch, *ibid.*, **27**, 178 (1899).

³⁵⁵ Riesser, *ibid.*, **49**, 210 (1906).

³⁵⁶ Clarke and Gillespie, *J. Am. Chem. Soc.*, **54**, 1964 (1932).

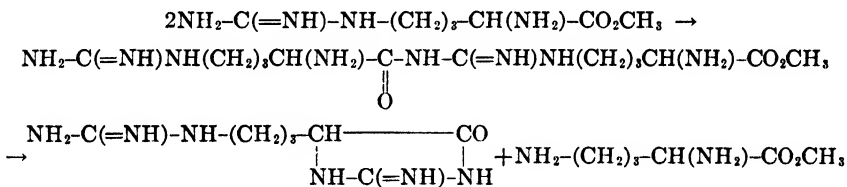
³⁵⁷ Bergmann and Köster, *Z. physiol. Chem.*, **159**, 179 (1926).

The piperidone derivative, when boiled with acids, readily undergoes hydrolysis to ornithine. The triacetyl anhydroarginine reacts not only with water, but with amines, which are thereby converted into derivatives of guanidine. Methylamine yields diacetylmethylguanidine, $\text{AcNH-C(=NAc)-NHCH}_3$, and glycine ester yields diacetyl glycoyamine ester, which on hydrolysis passes over into glycoyamidine.³⁵⁸

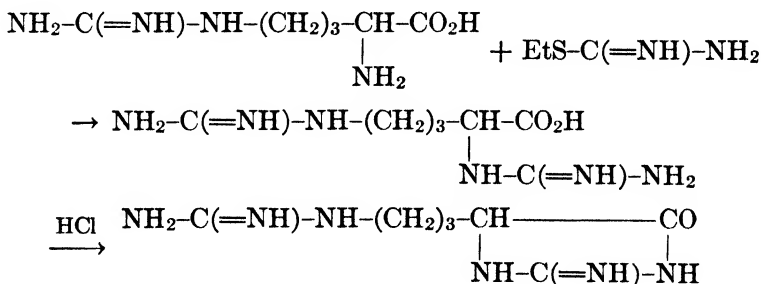


Creatinine is similarly formed from sarcosine ester.

When the methyl ester of guanidine is liberated from its dihydrochloride, it undergoes auto-condensation,³⁵⁹ yielding ornithine ester and an anhydride of α, δ -diguanidinovaleric acid³⁶⁰ containing a glyco-
cyamidine group. The reaction, which is analogous to that involved in the synthesis of glyco-
cyamidine from guanidine and glycine ester (p. 891), is explained as consisting in the condensation of the ester group of one molecule with the guanidino group of another, followed immediately by disproportionate cleavage.



The constitution of the diguanidinovaleric anhydride was confirmed by synthesis:



Like glycoamine and creatine, it gives a red color with alkaline picrate (p. 894).

³⁵⁸ Bergmann and Zervas, *ibid.*, **172**, 277 (1927); **173**, 80 (1928).

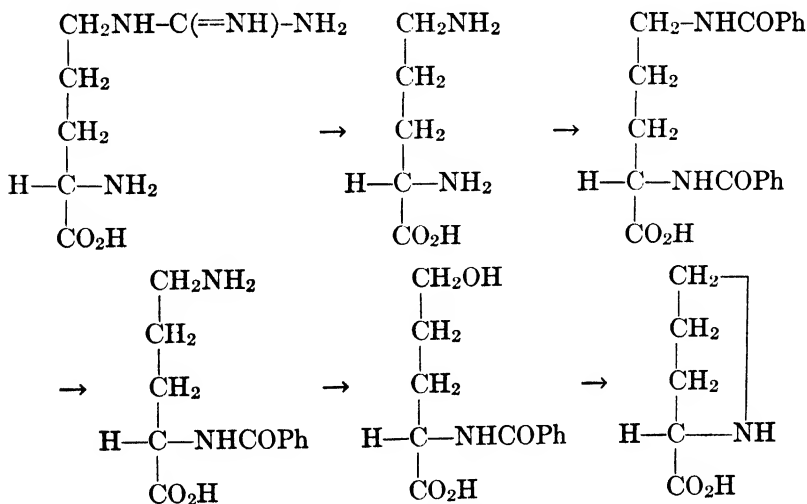
³⁵⁹ Fischer and Suzuki, *Ber.*, **38**, 4173 (1905).

³⁶⁰ Zervas and Bergmann, *Ber.*, **61**, 1195 (1928).

Natural arginine has the same configuration as the other natural amino acids;¹⁰ as it is dextrorotatory in acid solution, its systematic designation is *l*(+)-arginine. The levorotatory variety is not attacked by arginase and may therefore conveniently be prepared by the action of liver press-juice upon *dl*-arginine.³⁵⁵ On the other hand, arginase readily hydrolyses γ -guanidinobutyric acid to γ -aminobutyric acid and urea, but is without action on ϵ -guanidinocaproic acid.³⁶¹ The kinetics of arginase action is imperfectly understood, for although hydrolysis is partially inhibited by ornithine, addition of the other end product, urea, is without effect.³⁶²

Ornithine does not appear to be a constituent of proteins. However, its dibenzoyl derivative, ornithuric acid, occurs in the excreta of birds receiving benzoic acid in their diet.³⁶³ Hippuric acid, the principal form in which benzoic acid is eliminated by mammals, is not produced by chickens under these conditions, even when glycine is administered simultaneously.³⁶⁴ Similarly, the difuroyl derivative of ornithine is excreted by chickens receiving furfural, which gives rise to furoylglycine in rabbits and dogs.³⁶⁵

On alkaline hydrolysis, ornithuric acid loses the terminal benzoyl group. The *l*- α -monobenzoylornithine so prepared from natural arginine, on successive treatment with nitrous acid and hot hydriodic acid, is converted into *l*-proline.³¹⁸



³⁶¹ Thomas, *Z. physiol. Chem.*, **88**, 465 (1913).

³⁶² Gross, *ibid.*, **112**, 236 (1921).

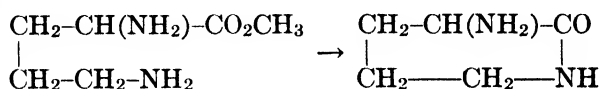
³⁶³ Jaffé, *Ber.*, **10**, 1925 (1877); Ellinger, *Z. physiol. Chem.*, **29**, 334 (1900).

³⁶⁴ Yoshikawa, *ibid.*, **68**, 79 (1910).

³⁶⁵ Jaffé and Cohn, *Ber.*, **20**, 2311 (1887); **21**, 3461 (1888).

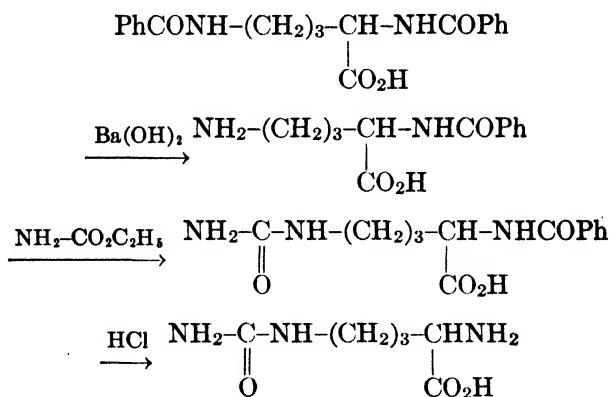
This series of reactions confirms the configurational identity of natural arginine, ornithine, and proline.

Ornithine has been synthesized by several general methods already discussed. The rather unstable free base has recently been obtained in a crystalline condition.³⁶⁶ Its properties closely resemble those of lysine, but the picrate is more soluble in water.³⁶⁷ It is converted into arginine by the addition of cyanamide.³⁶⁸ The methyl ester, when liberated from its hydrochloride, undergoes autocondensation, not to a diketopiperazine but to β -amino- α -piperidone,³⁶⁹



from which ornithine is regenerated on heating with hydrochloric acid.

Citrulline, isolated from watermelon juice in 1914 by Koga and Odake,³⁷⁰ was recognized as α -amino- δ -carbamidovaleric acid by Wada,³⁷¹ who confirmed its constitution by synthesis:



Citrulline is formed in small amounts from arginine by putrefaction³⁷² and by the action of *B. pyocyaneus*, which contains no arginase³⁷³ but to which a specific enzyme "arginine desimidase" is imputed. Its isolation from a tryptic digest of casein has been reported,³⁷⁴ with the sur-

³⁶⁶ Vickery and Cook, *J. Biol. Chem.*, **94**, 393 (1931).

³⁶⁷ Kossel and Weiss, *Z. physiol. Chem.*, **68**, 160 (1910).

³⁶⁸ Schulze and Winterstein, *Ber.*, **32**, 3191 (1899); *Z. physiol. Chem.*, **34**, 128 (1901).

³⁶⁹ Fischer and Zemplén, *Ber.*, **42**, 4878 (1909).

³⁷⁰ Koga and Odake, *J. Chem. Soc. Japan*, **35**, 519 (1914).

³⁷¹ Wada, *Biochem. Z.*, **224**, 420 (1930).

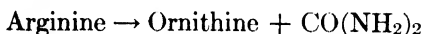
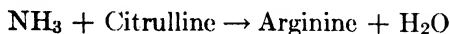
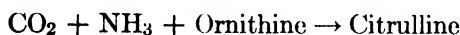
³⁷² Ackermann, *Z. physiol. Chem.*, **203**, 66 (1931).

³⁷³ Horn, *ibid.*, **216**, 244 (1933).

³⁷⁴ Wada, *Biochem. Z.*, **257**, 1 (1933).

prising assertion that it is converted into proline on heating with hydrochloric acid; δ -monobenzoylornithine is alleged to undergo the same change.

The relationship between arginine, ornithine, and citrulline is of extreme importance in the production of urea in the living body. Krebs and Henseleit³⁷⁵ have shown that the synthesis of urea, in the presence of intact liver tissue, from ammonia and the carbon dioxide resulting from the biochemical oxidation of glucose, lactic acid, or pyruvic acid, is markedly accelerated by the addition of any one of the above three amino acids. The process may be schematically expressed as follows:



The first and second reactions take place through the influence of some agency present in surviving liver tissue, for no urea synthesis occurs if the tissue be crushed; the third reaction is, of course, brought about by arginase.

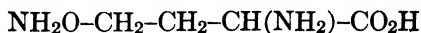
The enzyme urease, which promotes the hydrolysis of urea and is widely employed for its estimation and, indirectly, that of arginine (p. 925), is usually secured from jack bean meal. Aqueous-alcoholic extracts of this bean contain a basic amino acid, canavanine, $\text{C}_5\text{H}_{12}\text{O}_3\text{N}_4$, which, like arginine, yields half of its nitrogen as urea on treatment with liver extract. This amino acid³⁷⁶ responds to the ninhydrin test (p. 879) and gives up only one of its nitrogen atoms to nitrous acid in the Van Slyke procedure; it is therefore an α -amino acid. It is more stable than arginine towards barium hydroxide, forms a tribenzoyl derivative, yields no guanidine on oxidation with barium permanganate, and responds to neither the Sakaguchi (p. 924) nor the biacetyl (p. 925) test. It gives a characteristic red color with a solution of sodium nitroprusside which has been autoxidized by exposure to light.

Canaline, $\text{C}_4\text{H}_{10}\text{O}_3\text{N}_2$, formed together with urea by the action of the specific enzyme canavanase present in liver extract, contains one α -amino group and one nitrogen atom which does not react with nitrous acid. It forms a dibenzoyl derivative. It gives a red color with alkaline picrate (Jaffé test, p. 894) but none with autoxidized nitroprusside. On

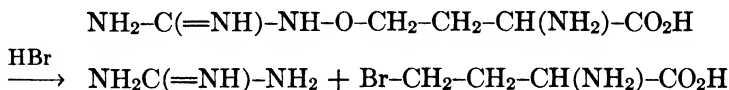
³⁷⁵ Krebs and Henseleit, *Z. physiol. Chem.*, **210**, 33 (1932).

³⁷⁶ Kitagawa and collaborators, *J. Biochem.*, (Japan), **11**, 265 (1929); **16**, 339 (1932); **18**, 333 (1933); **23**, 181; **24**, 407 (1936); [*C. A.*, **28**, 2678 (1934); **29**, 7280 (1935)].

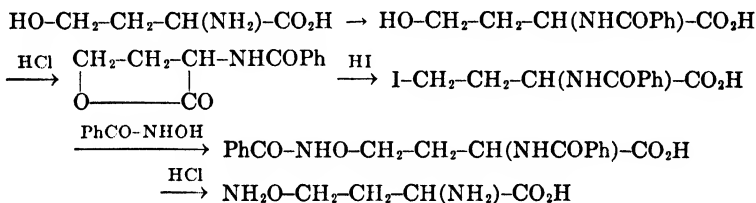
catalytic reduction it is converted into ammonia and α -amino- γ -hydroxybutyric acid;³⁷⁷ this points to the constitution:



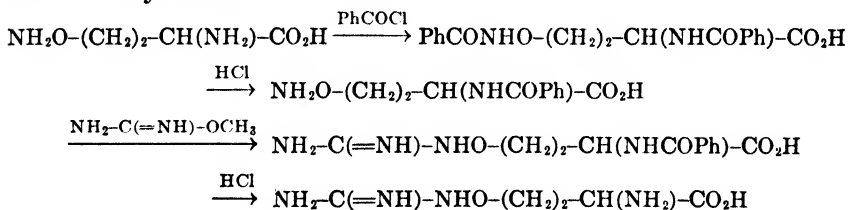
The reduction of the oxygen-nitrogen bond is analogous to that brought about by hydriodic acid with α -benzylhydroxylamine.³⁷⁸ Canavanine and dibenzoylcanaline do not respond to catalytic hydrogenation. On the other hand, by the action of hydrobromic acid canavanine yields α -amino- γ -bromobutyric acid and guanidine.³⁷⁹



Canaline has been regenerated from α -amino- γ -hydroxybutyric acid by condensing the γ -iodo- α -benzoylamino acid with benzhydroxamic acid.



The reconversion of canaline into canavanine has been effected by the use of methylisourea.



As is to be expected of an O-ether of hydroxylamine, canaline can form condensation products with aldehydes. An ethylidene derivative, which can be prepared from canaline and acetaldehyde, has been isolated from the products of the digestion of canavanine with liver extract.

The occurrence in nature of a derivative of hydroxyguanidine, a substance³⁸⁰ which has apparently received no attention for half a

³⁷⁷ Fischer and Blumenthal, *Ber.*, **40**, 106 (1907); Sørensen and Andersen, *Z. physiol. Chem.*, **56**, 250 (1908).

³⁷⁸ Meyer, *Ber.*, **16**, 167 (1883).

³⁷⁹ Gulland and Morris, *J. Chem. Soc.*, 763 (1935).

³⁸⁰ Prätorius-Seidler, *J. prakt. Chem.*, [2] **21**, 129 (1880).

century, warrants further study of the group. The titration curve of canavanine³⁸¹ indicates that the guanidinoxyl group is somewhat more strongly basic than the imidazole group of histidine but more weakly basic than the terminal amino group in lysine, which in turn is weaker than the guanidino group in arginine. The aminoxyl (NH₂O—) group in canaline is more weakly basic than even the imidazole.

From a study of analogous compounds, it appears that the color with autoxidized nitroprusside is characteristic of the guanidoxyl, that with picrate of the aminoxyl, group.

Histidine. Histidine, an almost universal constituent of proteins, was discovered simultaneously by Kossel³⁸² in the fraction precipitated by mercuric chloride from the hydrolysate of a protamine of fish roe, and by Hedin³⁸³ in the salt C₆H₇O₂N₂Ag₂·H₂O precipitated from protein hydrolysates on adding silver nitrate and alkali. Both processes have subsequently been developed into convenient preparative methods; the former is applicable when only histidine is desired,³⁸⁴ the latter when the preparation of all the "hexone bases" is necessary.³⁸⁵ Mercuric sulfate in 5 per cent sulfuric acid (Hopkins's reagent) affords a somewhat cleaner separation from arginine than mercuric chloride³⁸⁶ and has been recommended³⁸⁷ for the estimation of histidine in small samples of protein.

The imidazole (glyoxaline) group in histidine is a relatively weak base; the dihydrochloride, when dissolved in water, hydrolyzes to the monohydrochloride,³⁸⁸ and the free amino acid, unlike lysine and arginine, is extracted by butyl alcohol from aqueous solution at pH 7,¹⁶ together with the monoamino monocarboxylic acids. Histidine forms well-defined salts with one and with two molecules of picrolonic acid;³⁸⁹ the diflavinate is sparingly soluble and of use for isolation, but the monoflavinate is difficult to prepare;³⁹⁰ it also forms a sparingly soluble Reineckate³⁹¹ which crystallizes out with those of proline and hydroxyproline (pp. 899, 906). It is precipitated from acid solution by phosphotungstic acid but tends to redissolve in excess of the precipitant.³⁹²

³⁸¹ Tomiyama, *J. Biol. Chem.*, **111**, 45 (1935).

³⁸² Kossel, *Z. physiol. Chem.*, **22**, 176 (1896).

³⁸³ Hedin, *ibid.*, **22**, 191 (1896).

³⁸⁴ Hanke and Koessler, *J. Biol. Chem.*, **43**, 521 (1920).

³⁸⁵ Vickery and Leavenworth, *ibid.*, **78**, 627 (1928).

³⁸⁶ Kossel and Patten, *Z. physiol. Chem.*, **38**, 39 (1903).

³⁸⁷ Rosedale and da Silva, *Biochem. J.*, **26**, 369 (1932).

³⁸⁸ Abderhalden and Einbeck, *Z. physiol. Chem.*, **62**, 322 (1909).

³⁸⁹ Brigl, *ibid.*, **64**, 337 (1910).

³⁹⁰ Vickery, *J. Biol. Chem.*, **71**, 303 (1926).

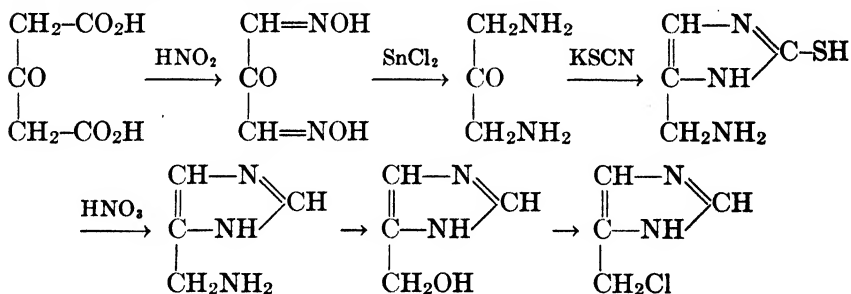
³⁹¹ Kapfhammer and Spörer, *Z. physiol. Chem.*, **173**, 245 (1928).

³⁹² Fränkel, *Monatsh.*, **24**, 229 (1903).

The constitution of histidine was established by Pauly,²¹¹ whose discovery that it yields an azo color with diazobenzenesulfonic acid forms the basis of a widely employed method for its colorimetric estimation.³⁹³ The imidazole nucleus accepts either one³⁹⁴ or two³⁹⁵ azo groups. The chief interfering substance in a protein hydrolysate is tyrosine; the ability of this to couple can be inhibited by benzylation, which under suitable conditions is without effect on the Pauly reaction for histidine.³⁹⁶ Histidine also survives treatment with nitric acid, which nitrates tyrosine and so prevents it from coupling;³⁹⁷ this procedure, however, suffers from the disadvantage of introducing a yellow color into the mixture to be tested. The most satisfactory procedure is to apply the Pauly test to a fraction previously precipitated by phosphotungstic acid or by silver.

A red color is formed on adding bromine water to a solution of histidine and changes to purple on addition of ammonia. This color reaction has been developed for the quantitative estimation of histidine, for which it appears to be specific.³⁹⁸ A transient red-violet color is developed on treating histidine with hydrogen peroxide in presence of a ferrous salt,³⁹⁹ presumably with the intermediate formation of β -imidazoleacetaldehyde.

Histidine has been synthesized⁴⁰⁰ by condensing the sodium derivative of ethyl chloromalonate with 4-chloromethylimidazole, prepared from α,γ -diaminoacetone,



the product being hydrolyzed, decarboxylated, and treated with ammonia.

³⁹³ Jorpes, *Biochem. J.*, **26**, 1507 (1932).

³⁹⁴ Wallach, Rung, and Behrend, *Ann.*, **271**, 28 (1892).

³⁹⁵ Pauly, *Z. physiol. Chem.*, **94**, 284 (1915).

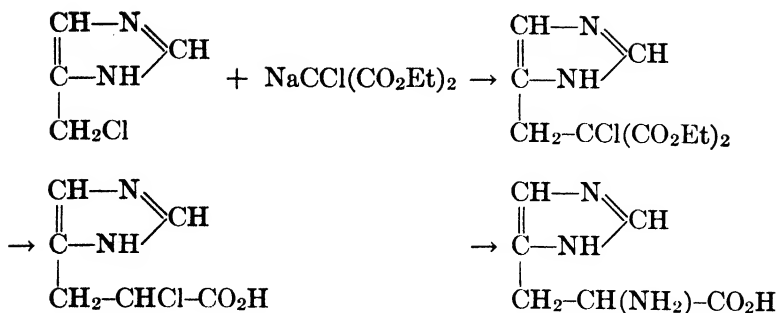
³⁹⁶ Inouye, *ibid.*, **83**, 79 (1913).

³⁹⁷ Brunswik, *ibid.*, **127**, 268 (1923).

³⁹⁸ Knoop, *Beitr. chem. Physiol. Path.*, **11**, 356 (1908); Kapeller-Adler, *Biochem. Z.*, **264**, 131 (1933).

³⁹⁹ Kikkoji and Neuberger, *ibid.*, **30**, 523 (1909).

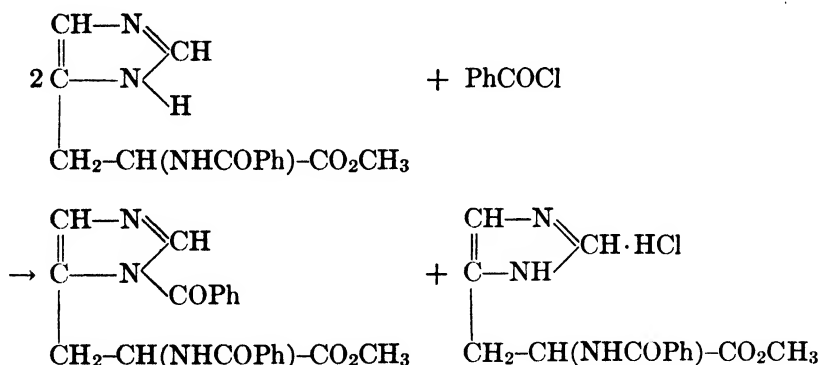
⁴⁰⁰ Pyman, *J. Chem. Soc.*, **99**, 668, 1386 (1911); **109**, 186 (1916).



The racemic histidine was resolved by crystallizing the *d*-tartrate. It has also been synthesized by the Erlenmeyer process from imidazolealdehyde and hippuric acid.

Two of the nuclear hydrogen atoms of histidine are replaceable by iodine, when the α -amino group is previously protected by benzylation.⁴⁰¹

The α -monobenzoyl derivative is formed with one molecular proportion of benzoyl chloride in presence of benzene and the minimally practicable amount of aqueous alkali; a benzoyl group can be introduced into the imidazole nucleus⁴⁰² by treating the methyl ester of α -benzoyl-histidine in benzene solution with a semi-molecular proportion of benzoyl chloride.



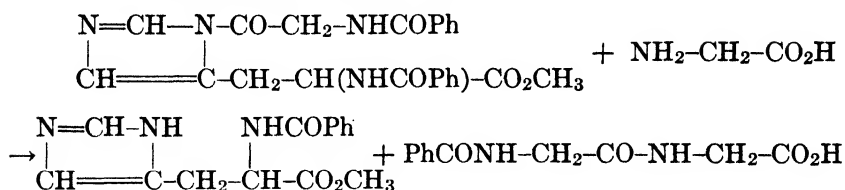
An interesting property of such esters of diacyl histidines is their ability to transfer the nuclear acyl group to amino acids or esters⁴⁰³ and presumably to other amines. For example, hippuryl chloride reacts with

⁴⁰¹ Pauly, *Ber.*, **43**, 2243 (1910).

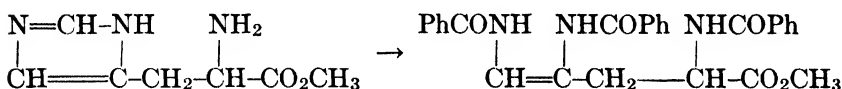
⁴⁰² Gerngross, *Z. physiol. Chem.*, **108**, 50 (1919).

⁴⁰³ Bergmann and Zervas, *ibid.*, **175**, 145 (1928).

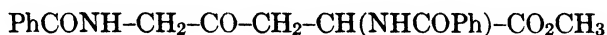
α -benzoylhistidine methyl ester to form a product which with glycine yields α -benzoylhistidine ester and hippurylglycine.



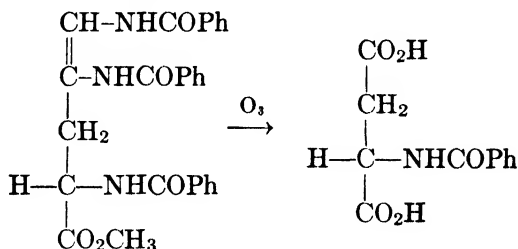
When histidine methyl ester is treated with benzoyl chloride in presence of aqueous sodium carbonate, the imidazole ring is opened.⁴⁰⁴



The product on treatment with methyl-alcoholic hydrogen chloride is converted to the methyl ester of γ -keto-ornithuric acid.⁴⁰⁵



On treatment with ozone, followed by partial hydrolysis, the tribenzoyl compound prepared from natural histidine is converted into the benzoyl derivative of natural *l*(-)-aspartic acid.⁴⁰⁶



Histidine of protein origin therefore possesses the same configuration as the other natural amino acids, as is also indicated by *pH*-dependence curves.¹⁰

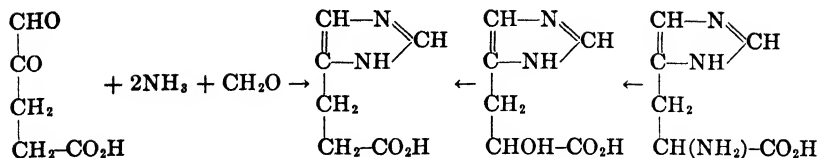
On treatment with nitrous acid, histidine yields β -imidazolelactic acid, which is reduced by phosphorus with hydriodic acid to β -imidazolepropionic acid,⁴⁰⁷ identical with that prepared from β -glyoxylpropionic acid with formaldehyde and ammonia.

⁴⁰⁴ Kossel and Edlbacher, *ibid.*, **93**, 396 (1915).

⁴⁰⁵ Langenbeck and Hutschenreuter, *ibid.*, **182**, 305 (1929).

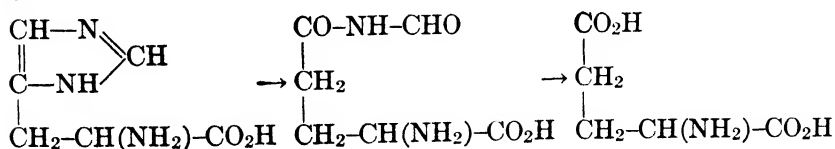
⁴⁰⁶ Langenbeck, *Ber.*, **58**, 227 (1925).

⁴⁰⁷ Knoop and Windaus, *Beitr. chem. Physiol. Path.*, **7**, 144 (1906).



β -Imidazolelactic acid, on oxidation by nitric acid followed by hydrogen peroxide, is successively converted into imidazoleglyoxylic acid and imidazolecarboxylic acid,⁴⁰⁸ a series of reactions which served to confirm the assignment of the primary amino group to the α -position.

Histidine is converted into β -imidazolelactic acid by some bacteria; others, particularly certain of the *B. coli* group, cause decarboxylation to histamine,⁴⁰⁹ a conversion which seems also to be brought about by ultra-violet light. A fungus, *Oidium lactis*, brings about reductive deamination to β -imidazolepropionic acid,⁴¹⁰ and certain micro-organisms (e.g., *B. paratyphosus* B) effect deamination without reduction to urocanic acid.⁴¹¹ This last product, identified by Hunter⁴¹² as β -imidazoleacrylic acid, is a form in which orally administered histidine is eliminated by dogs.⁴¹³ However, since less than half of the histidine can be recovered from the urine as imidazole derivatives,⁴¹⁴ some profound decomposition must take place in the body. A reaction of this type is brought about by an enzyme, histidase, present in the liver. Under its influence *l*-histidine breaks down into *l*(+)-glutamic acid and two moles of ammonia,⁴¹⁵ a hydrolytic converse of the classical synthesis of imidazoles from glyoxals, formaldehyde, and ammonia. Indirect evidence points to the intermediate formation of a formylated glutamine.



Histidine cannot be synthesized in the mammalian body from simpler compounds, but is apparently so produced from β -imidazolelactic acid and imidazolepyruvic acid, which stimulate growth on diets deficient in

⁴⁰⁸ Knoop, *ibid.*, **10**, 111 (1907).

⁴⁰⁹ Hanke and Koessler, *J. Biol. Chem.*, **50**, 131 (1922); Hirai, *Biochem. Z.*, **267**, 1 (1933).

⁴¹⁰ Kiyokawa, *Z. physiol. Chem.*, **214**, 38 (1933).

⁴¹¹ Raistrick, *Biochem. J.*, **11**, 71 (1917).

⁴¹² Hunter, *J. Biol. Chem.*, **11**, 537 (1912).

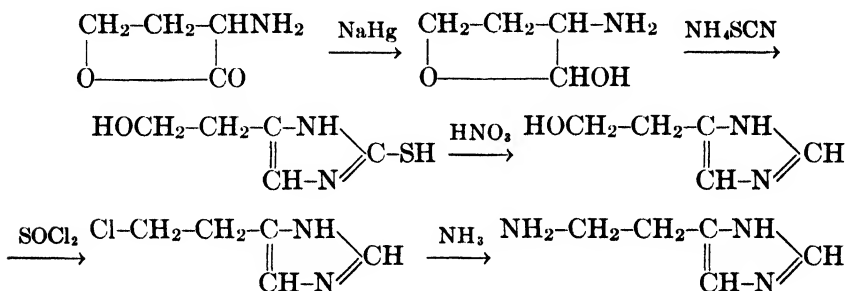
⁴¹³ Kotake and Konishi, *Z. physiol. Chem.*, **122**, 230 (1922).

⁴¹⁴ Abderhalden and Buadze, *ibid.*, **200**, 87 (1931).

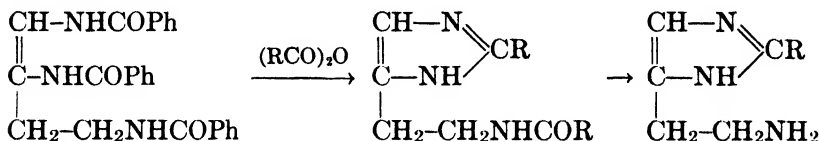
⁴¹⁵ Edlbacher and collaborators, *ibid.*, **191**, 225 (1930); **224**, 261 (1934).

histidine but otherwise adequate.⁴¹⁶ Urocanic acid, on the other hand, is almost without effect on growth.

Histamine, the decarboxylation product of histidine, is of great physiological interest, on account of its powerful vasodilator action. It is present in lung, liver, muscle, and blood.⁴¹⁷ Lung and kidney contain a highly specific enzyme, histaminase, which breaks down histamine,⁴¹⁸ much as histidase disrupts *l*-histidine. Histamine has been synthesized by reduction of imidazoleacetonitrile⁴⁰⁰ and from α -aminobutyrolactone³⁷⁷ by the following steps:⁴¹⁹



On treatment with benzoyl chloride and alkali the imidazole ring is opened, as in the case of histidine methyl ester; the product, on heating with acid anhydrides and hydrolyzing, yields physiologically inactive 2-alkyl homologs of histamine.⁴²⁰



Although 2-thiolhistidine has never been isolated from natural sources, certain proteins, notably zein, give a positive color reaction for thiolimidazoles.⁴²¹ Thiolhistidine has been synthesized by the action of thiocyanate upon γ -ketoörrnithine, prepared either from histidine (cf. p. 936), or from aspartic acid.⁴²²

⁴¹⁶ Harrow and Sherwin, *J. Biol. Chem.*, **70**, 683 (1926).

⁴¹⁷ Best, Dale, Dudley, and Thorpe, *J. Physiol.*, **62**, 397 (1926); Thorpe, *Biochem. J.*, **22**, 94 (1928); Barsoum and Gaddum, *J. Physiol.*, **85**, 1 (1935).

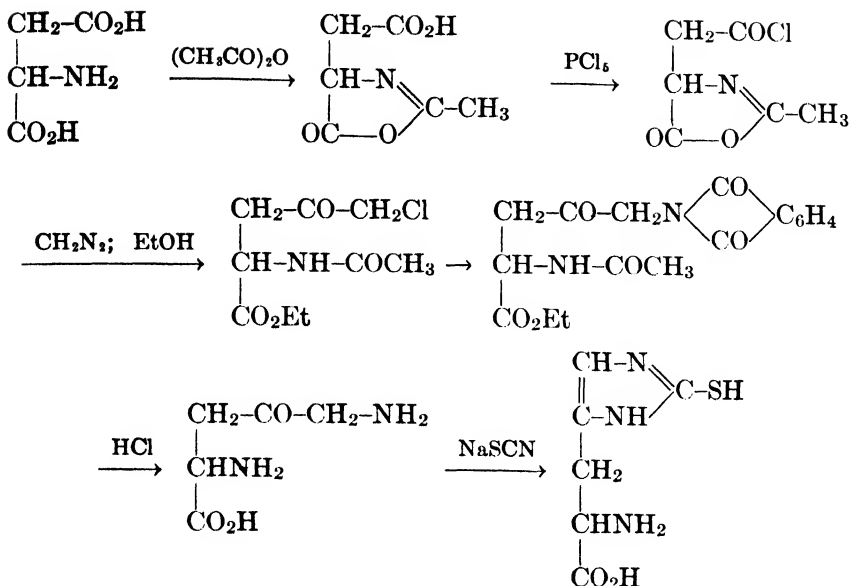
⁴¹⁸ McHenry and Gavin, *Biochem. J.*, **26**, 1365 (1932).

⁴¹⁹ Garforth and Pyman, *J. Chem. Soc.*, 489 (1935).

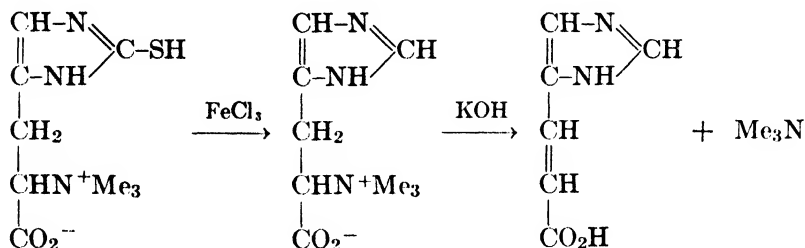
⁴²⁰ van der Merwe, *Z. physiol. Chem.*, **177**, 301 (1928).

⁴²¹ Eagles and Vars, *J. Biol. Chem.*, **80**, 615 (1928).

⁴²² Ashley and Harington, *J. Chem. Soc.*, 2586 (1930); Harington and Overhoff, *Biochem. J.*, **27**, 338 (1933).



Ergothioneine, the trimethylbetaine of thiohistidine, is present in ergot and in blood. Attempts to synthesize it by methylating thiohistidine have failed, owing to the breakdown of the methylated product into trimethylamine and an unsaturated acid. In the same way, histidine trimethylbetaine, formed by oxidizing ergothioneine, breaks down on treatment with alkali⁴²³ into trimethylamine and urocanic acid.



Beef muscle contains a water-soluble base called carnosine⁴²⁴ which, like histidine, is precipitated by mercuric sulfate and from alkaline solution by silver nitrate, and yields an azo color with diazobenzenesulfonic acid; it gives no color, however, with bromine,⁴²⁵ and its Reineckate is less soluble than that of histidine.⁴²⁶ On alkaline

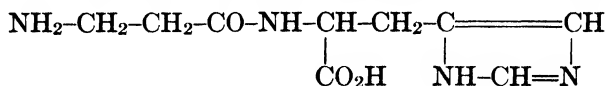
⁴²³ Barger and Ewins, *J. Chem. Soc.*, **99**, 2336 (1911).

⁴²⁴ Gulewitsch and Amiradzibi, *Z. physiol. Chem.*, **30**, 565 (1900).

⁴²⁵ Hunter, *Biochem. J.*, **16**, 640 (1922).

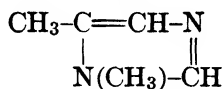
⁴²⁶ Smorodintsev, *Biochem. Z.*, **222**, 425 (1930).

hydrolysis it yields *l*-histidine,⁴²⁷ and its phenylureide on boiling with acids breaks down into histidine and the phenylureide of β -aminopropionic acid.⁴²⁸ Carnosine is therefore β -aminopropionylhistidine.

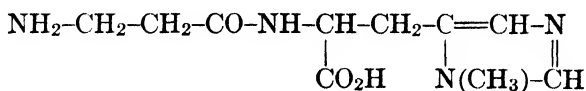


Synthetically it has been obtained, in poor yields, by the action of ammonia upon β -iodopropionylhistidine⁴²⁹ and by reduction of β -nitropropionylhistidine.⁴³⁰ A practical synthesis has recently been developed:⁴³¹ β -aminopropionic acid is condensed with benzyl chloro-carbonate; the resulting carbobenzoxy- β -alanine is converted successively into its chloride, methyl ester, hydrazide, and azide (*cf.* p. 887); this is coupled with histidine methyl ester, and, after hydrolysis, the carbobenzoxy group is removed with palladium and hydrogen.

A methyl homolog of carnosine, called anserine,⁴³² occurs, together with carnosine, in the skeletal muscles of various mammals, fish, reptiles, and birds.⁴³³ It is not precipitated by silver and does not couple with diazo compounds, but is thrown down by mercuric sulfate. On hydrolysis it yields β -alanine and a methylhistidine;⁴³⁴ on heating with soda-lime it yields 1,5-dimethylimidazole,⁴³⁵



a reaction which establishes the position of the nuclear methyl group. In other respects it closely resembles carnosine, and its properties are in full accord with the structure:



Tryptophan. Tryptophan is a nearly general protein constituent, but is absent, or nearly so, from gelatin, zein, and insulin. It does not appear among the products of acid hydrolysis of proteins, as it is converted by the action of mineral acids into humins, formed by condensa-

⁴²⁷ Gulewitsch, *Z. physiol. Chem.*, **50**, 535 (1907).

⁴²⁸ Abderhalden and Geidel, *Fermentforschung*, **12**, 518 (1931).

⁴²⁹ Baumann and Ingvaldsen, *J. Biol. Chem.*, **35**, 263 (1918).

⁴³⁰ Barger and Tutin, *Biochem. J.*, **12**, 402 (1918).

⁴³¹ Sifferd and du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

⁴³² Ackermann, Timpe, and Poller, *Z. physiol. Chem.*, **183**, 1 (1929).

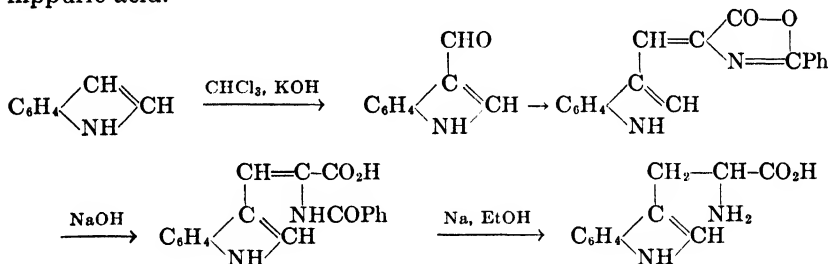
⁴³³ Wolff and Wilson, *J. Biol. Chem.*, **109**, 565 (1935).

⁴³⁴ Linneweh and collaborators, *Z. physiol. Chem.*, **183**, 11 (1929); **189**, 80 (1930).

⁴³⁵ Keil, *ibid.*, **187**, 1 (1930).

tion with aldehydes derived from the protein or substances, such as carbohydrates, associated with it.⁴³⁶ Tryptophan is also rapidly destroyed when proteins are hydrolyzed with boiling sodium hydroxide solution, but it is stable to barium hydroxide, which, however, brings about racemization. For the preparation of the natural, optically active product, it is customary to resort to enzymatic hydrolysis by means of trypsin—a slow procedure, which liberates less than half of the tryptophan.⁴³⁷ The precipitate, characteristic of tryptophan, which forms on addition of mercuric sulfate to the tryptic digest containing 5 per cent of free sulfuric acid,⁴³⁸ contains peptides from which tryptophan is released on further tryptic digestion. Tryptophan is extracted by butyl alcohol from neutral solution; this fact has been usefully applied, for preparative purposes, to the fraction precipitated by mercury.⁴³⁹

Natural tryptophan is levorotatory and possesses the same configuration as other amino acids.¹⁰ The racemic variety has been synthesized by the Erlenmeyer procedure from 3-indolealdehyde and hippuric acid.⁴⁴⁰



Better yields are obtainable by condensing the aldehyde with hydantoin in presence of piperidine and heating the resulting indolalhydantoin with ammonium sulfide⁴⁴¹ (cf. p. 888).

Colored products are formed on treating tryptophan with aldehydes in presence of concentrated mineral acids. The violet color developed with glyoxylic acid in sulfuric acid, which aided Hopkins and Cole in the discovery of tryptophan, is rendered more certain and sensitive by the addition of a trace of copper.⁴⁴² This probably acts as an oxygen

⁴³⁶ Burr and Gortner, *J. Am. Chem. Soc.*, **46**, 1224 (1924).

⁴³⁷ Onslow, *Biochem. J.*, **15**, 383, 392 (1931).

⁴³⁸ Hopkins and Cole, *J. Physiol.*, **27**, 418 (1902).

⁴³⁹ Cox and King, "Organic Syntheses," John Wiley and Sons, New York (1930) Vol. 10, p. 100.

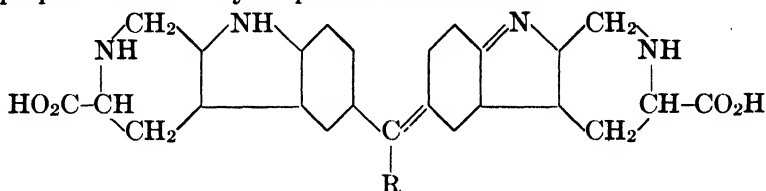
⁴⁴⁰ Ellinger and Flamand, *Z. physiol. Chem.*, **55**, 8 (1908).

⁴⁴¹ Boyd and Robson, *Biochem. J.*, **29**, 2256 (1935).

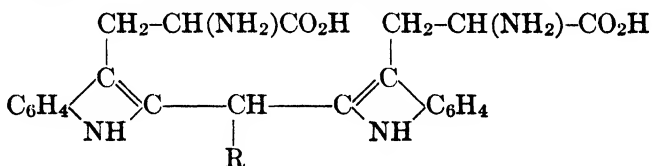
⁴⁴² Winkler, *Z. physiol. Chem.*, **228**, 50 (1934).

carrier, for with other aldehydes the presence of oxidizing agents has been found necessary for the development of a blue color suitable for quantitative purposes. Especial use has been made of vanillin,⁴⁴³ *p*-dimethylaminobenzaldehyde,⁴⁴⁴ and formaldehyde,⁴⁴⁵ with hydrogen peroxide and nitrous acid as oxidants. In the absence of the latter, red to violet tints of indeterminate hue are formed.

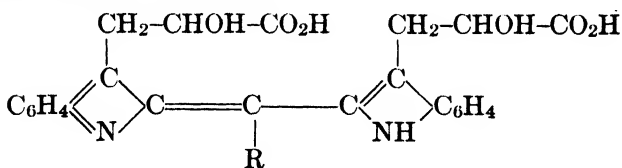
It has been suggested⁴⁴⁶ that the blue pigments produced from tryptophan with aldehydes possess the structure:



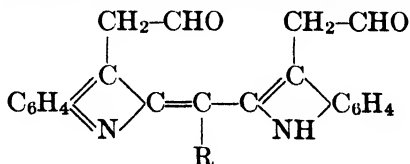
and that in the red intermediates the side chain of the tryptophan has not cyclized. According to another view,⁴⁴⁷ cyclization of the side chain does not occur; the hypothetical primary product is,



which with nitrous acid yields,



and with hydrogen peroxide the corresponding aldehyde.



This view is supported by the nitrogen content of the products, which were, however, not secured in crystalline condition.

⁴⁴³ Ragins, *J. Biol. Chem.*, **80**, 543 (1928).

⁴⁴⁴ Boyd, *Biochem. J.*, **23**, 78 (1929).

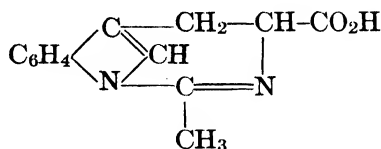
⁴⁴⁵ Fürth and collaborators, *Biochem. Z.*, **109**, 103, 124 (1920).

⁴⁴⁶ Fearon, *Biochem. J.*, **14**, 548 (1920).

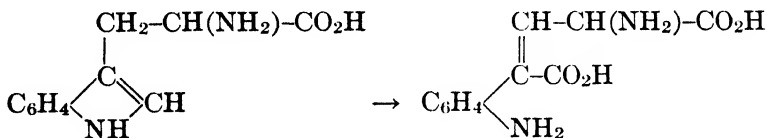
⁴⁴⁷ Ghigi, *Gazz. chim. ital.*, **63**, 411 (1933).

Like tyrosine, tryptophan yields a yellow color with nitric acid⁴⁴⁸ and a blue color with Folin's phenol reagent.²¹⁴ Both these colorimetric tests have been applied quantitatively, as has the red color, extractable by amyl alcohol, produced with bromine water.⁴⁴⁹

Whereas most amino acids when heated with a mixture of acetyl chloride and acetic acid are converted into derivatives of oxazole (p. 873), tryptophan yields a product of a different type⁴⁵⁰ in which the indole nitrogen atom is involved in ring formation.

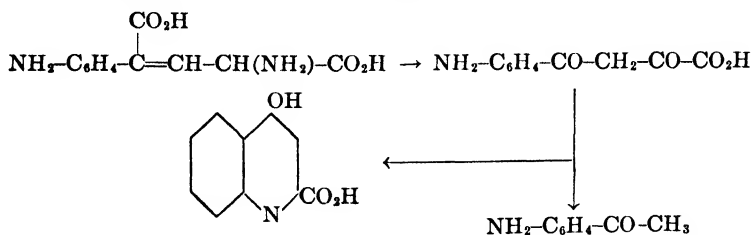


When tryptophan is administered to normal rabbits, it is partially eliminated as γ -hydroxyquinoline- α -carboxylic (kynurenic) acid. This compound is also formed, though in lower yields, from indole-3-pyruvic acid.⁴⁵¹ Rabbits on a diet of polished rice, which is deficient in thiamin (vitamin B₁), excrete not only kynurenic acid but also an amino acid, kynurenine, in which the pyrrole ring of tryptophan has ruptured.⁴⁵²



This reaction takes place only with *l*-tryptophan, not with the "unnatural" variety.

Kynurenine is stable to acids but is unstable in alkaline solution, yielding ammonia, carbon dioxide, *o*-aminoacetophenone and kynurenic acid, apparently by way of *o*-aminobenzoylpyruvic acid.



⁴⁴⁸ Tillmans, Hirsch, and Stoppel, *Biochem. Z.*, **198**, 379 (1928).

⁴⁴⁹ Levene and Rouiller, *J. Biol. Chem.*, **2**, 481 (1907).

⁴⁵⁰ Wrede and Feuerriegel, *Ber.*, **66**, 1073 (1933).

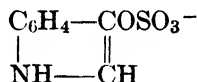
⁴⁵¹ Ellinger and Matsuoka, *Z. physiol. Chem.*, **109**, 259 (1920).

⁴⁵² Kotake and collaborators, *ibid.*, **195**, 139 (1931); **214**, 1 (1933).

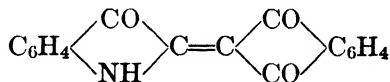
Kynurenine is also converted into kynurenic acid on administration to dogs, the reaction taking place in the liver.

Similar conversions are brought about by certain microorganisms; *B. subtilis*, for instance, forms kynurenine, kynurenic acid, and anthranilic acid from tryptophan. Of other organisms, some (*B. coli*) break down *l*-tryptophan into indole in the presence, and into indole-3-propionic acid in the absence, of air;⁴⁵³ some (*B. aminophilus intestinalis*) effect decarboxylation to β -indoleethylamine;⁴⁵⁴ some (*Proteus* and *Oidium lactis*, respectively) bring about deamination to varieties of indolelactic acid having opposite configurations;⁴⁵⁵ some (yeast) cause both deamination and decarboxylation to tryptophol (β -indole-ethanol).⁴⁵⁶

In intestinal putrefaction, tryptophan is broken down into indole and β -methylindole (skatole). These products partly diffuse into the blood stream, where they undergo further chemical alteration. Little is known about the fate of skatole; it is possibly oxidized at the side chain. Indole is oxidized to indoxyl, which is eliminated by the kidney as a sulfuric ester, indican.



The presence in the urine of abnormally large amounts of indoxylsulfuric acid, diagnostic of chronic constipation or intestinal obstruction, is indicated by the production of indigo on treatment with oxidizing agents.⁴⁵⁷ Indican can be estimated colorimetrically by treatment with triketohydrindene,⁴⁵⁸ which yields a red condensation product.



In living mammals, an appreciable part of the tryptophan taken in the normal diet is utilized for protein synthesis. Normal growth is impossible on diets deficient in this essential amino acid, but can be induced by the addition, to such diets, of not only *l*-tryptophan, but *d*-tryptophan or acetyl-*l*-tryptophan. The effect of a large number of derivatives of tryptophan on growth and kynurenic acid production is summarized in the accompanying table. From the results it appears

⁴⁵³ Woods, *Biochem. J.*, **29**, 640, 649 (1935).

⁴⁵⁴ Berthelot and Bertrand, *Compt. rend.*, **154**, 1826 (1912).

⁴⁵⁵ Sasaki and Otsuka, *Biochem. Z.*, **121**, 167 (1921).

⁴⁵⁶ Ehrlich, *Ber.*, **45**, 883 (1912).

⁴⁵⁷ Salkowski, *Z. physiol. Chem.*, **42**, 213 (1904).

⁴⁵⁸ Kumon, *ibid.*, **231**, 205 (1935).

	GROWTH STIMULATION	KYNURENIC ACID FORMATION	LITERATURE REFERENCES
<i>l</i> -Tryptophan.....	+	+	
<i>d</i> -Tryptophan.....	+	—	459, 460
Acetyl- <i>l</i> -tryptophan.....	+	±	459, 460
Acetyl- <i>d</i> -tryptophan.....	—	—	459, 460
Propionyl- <i>l</i> -tryptophan.....	+	..	461
Benzoyl- <i>l</i> -tryptophan.....	—	..	462
Phenacetyl- <i>l</i> -tryptophan.....	—	..	461
<i>l</i> -Tryptophan esters.....	+	..	461, 462
<i>l</i> -Tryptophan amides.....	+	+	463
(+)-Indolelactic acid.....	+	+	452, 464
(-)-Indolelactic acid.....	—	—	452, 464
Indolepyruvic acid.....	+	+	451, 452
Indolepyruvic acid oxime.....	—	—	464
Indolepropionic acid.....	—	..	465
Indoleacrylic acid.....	—	—	464, 466
Kynurenine.....	—	+	452, 467

that the biochemical mechanisms respectively involved are different and independent. The behavior of the two forms of acetyltryptophan indicates that the body can hydrolyze the *l* variety specifically, but apparently at so slow a rate that very little of the resulting tryptophan is diverted from its major function—that of growth stimulation—to the production of kynurenic acid. On the other hand, hydrolysis of the amides ($-\text{NH}_2$, $-\text{NHEt}$, $-\text{NEt}_2$, $-\text{NHPh}$, $-\text{NEtPh}$) of *l*-tryptophan seems to be more rapidly accomplished, as all stimulate growth and produce kynurenic acid more freely than acetyl-*l*-tryptophan, though not so readily as *l*-tryptophan. The fact that indolepyruvic acid can also without undue difficulty fulfill both functions of tryptophan, whereas *d*-tryptophan yields little or no kynurenic acid, may likewise be regarded as indicating a relatively slow conversion of *d*-tryptophan into *l*-tryptophan through the pyruvic acid. However, interpretation of the data is complicated by the circumstance that the growth studies were carried out with diets devoid of tryptophan, which was not true of the experiments on the production of kynurenic acid. In the absence of an urgent demand for tryptophan it is conceivable that a related

⁴⁵⁹ du Vigneaud, Sealock, and Van Etten, *J. Biol. Chem.*, **98**, 565 (1932).

⁴⁶⁰ Berg, *ibid.*, **104**, 373 (1934).

⁴⁶¹ Berg and Hanson, *Proc. Iowa Acad. Sci.*, **41**, 165 (1934) [*C. A.*, **29**, 4049 (1935)].

⁴⁶² Berg, *J. Biol. Chem.*, **91**, 513 (1931).

⁴⁶³ Bauguess and Berg, *ibid.*, **106**, 615 (1934).

⁴⁶⁴ Bauguess and Berg, *ibid.*, **104**, 675, 691 (1934).

⁴⁶⁵ Jackson, *ibid.*, **84**, 1 (1929).

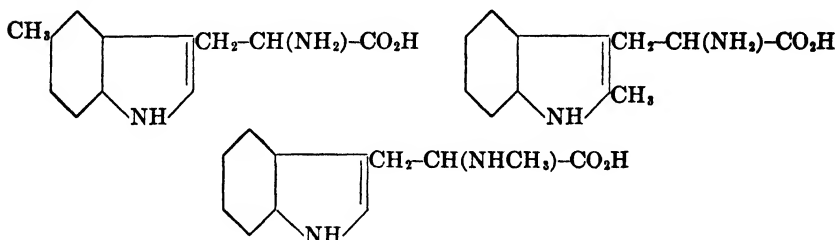
⁴⁶⁶ Bauguess and Berg, *Proc. Iowa Acad. Sci.*, **40**, 110 (1933) [*C. A.*, **29**, 2579 (1935)].

⁴⁶⁷ Jackson and Jackson, *J. Biol. Chem.*, **96**, 697 (1932).

compound such as indolepyruvic acid may be metabolized more extensively by oxidative degradation than by amination to *l*-tryptophan.

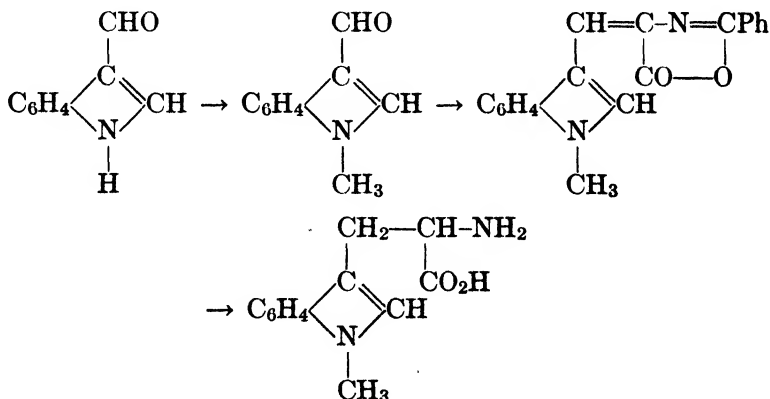
The behavior of the two varieties of indolelactic acid is of interest: the body apparently possesses an enzyme capable of converting the dextrorotatory, but not the levorotatory, variety into the keto acid, and it has been suggested⁴⁵² that the dextrorotatory form has the same configuration as *l*-tryptophan, from which it is produced by *Proteus* but not by *Oidium lactis* (cf. methionine, p. 920). Finally, it is clear that, while kynurenine is an intermediate in the production of kynurenic acid, it cannot be biochemically converted into tryptophan.

A study of three methyl-*dl*-tryptophans,



has shown⁴⁶⁸ that the power to stimulate growth is not impaired by methylation of the α -nitrogen atom of tryptophan, but is completely inhibited by introduction of a methyl group into either nucleus of the indole structure.

A methyltryptophan in which the pyrrole-nitrogen atom is methylated has been synthesized⁴⁶⁹ from indolealdehyde by methylation followed by the Erlenmeyer procedure, sodium lead alloy being employed for reduction of the azlactone.

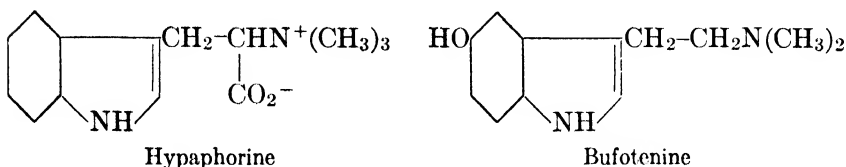


⁴⁶⁸ Gordon and Jackson, *ibid.*, **110**, 151 (1935).

⁴⁶⁹ Wieland, Konz, and Mittasch, *Ann.*, **513**, 1 (1934).

The product is precipitable from acid solution by mercuric sulfate, but fails to give the characteristic color tests with glyoxylic acid and with *p*-dimethylaminobenzaldehyde.

The trimethylbetaine of *l*-tryptophan, hypaphorine, has been isolated from the seeds of a Javanese tree.⁴⁷⁰ A closely related compound, bufotenine,



is present in the venomous secretion of the European toad.⁴⁶⁹

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⁴⁷⁰ v. Romburgh and Barger, *J. Chem. Soc.*, **99**, 2068 (1911).

CHAPTER 11

THE CHEMISTRY OF PYRIMIDINES, PURINES AND NUCLEIC ACIDS *

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* For personal assistance in the compilation of the material discussed in this chapter, the author is indebted to the kind coöperation of the following associates: Dr. John J. Donleavy, Assistant Professor of Chemistry, Yale University; Dr. Elizabeth Dyer, Instructor in Chemistry, University of Delaware, Newark, Delaware; Dr. Werner Bergmann, Senior Research Fellow of the Textile Foundation, Inc., in Yale University, 1934-35; and Dr. Karl Folkers, Research Chemist, Merck and Company, Rahway, New Jersey. For a recent review of "Pyrimidines: Their Amino and Aminoöxy Derivatives," including a complete reference and author index, the reader should consult the paper by Johnson and Hahn, published in *Chem. Rev.*, **13**, 193-303 (1933).

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INTRODUCTION

The method of treatment of the chemistry of pyrimidines, purines, and nucleic acids presented in this chapter has been adapted to meet the interests and requirements of advanced students in organic chemistry. The contribution is not to be regarded as a catalog of synthetic products and processes in the pyrimidine and purine fields, nor has it been constructed as a laboratory guide to the preparation of organic compounds belonging to these two series.

A new chemistry of pyrimidines and purines has been developed since Liebig and Wöhler's classical researches on uric acid. The work accomplished in this field of heterocyclic chemistry has been a most valuable contribution to our knowledge of the chemistry of cyclic ureides. Notwithstanding the great advances made by organic chemists in their contributions to biochemistry in this field, when it comes to the fundamental question of the mode of origin of these natural products in animal and plant organisms, it must be confessed that up to the present time very little progress has been made.

NOMENCLATURE

Pyrimidine^{1,2} is expressed structurally as a heterocyclic ring containing four carbon and two nitrogen atoms, and, according to the system of nomenclature used in Richter's "Lexikon" and in *Chemical Abstracts*,³ it is classified among the *Azines*. It is called a *meta*-diazine or 1,3-diazine.⁴

Diazines	$\left\{ \begin{array}{l} 1,2\text{-diazine or pyridazine} \\ 1,3\text{-diazine or pyrimidine} \\ 1,4\text{-diazine or pyrazine} \end{array} \right.$
--------------------	---

Graphically the pyrimidine cycle⁵ may be expressed by a hexagonal structure (pyrimidine B) as represented by formula II. This formula shows the structural relationship of pyrimidine to the azine *pyridine* (III). The numbering of the positions in the cycle is in accordance with the system used in Richter's "Lexikon."⁶ The

¹ Pinner, *Ber.*, **17**, 2519 (1884); **18**, 759 (1885).

² Gabriel, *Ber.*, **33**, 3666 (1900).

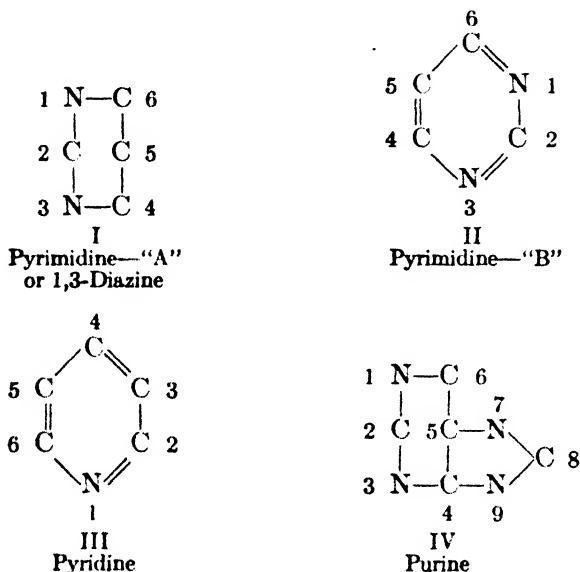
³ The third Decennial Index of *Chemical Abstracts* is to have the nomenclature now used in *C. A.*

⁴ Consult "A System of Organic Nomenclature," Patterson and Curran, *J. Am. Chem. Soc.*, **39**, 1623 (1917).

⁵ Pinner, *Ber.*, **18**, 759 (1885).

⁶ Richter, "Lexikon der Kohlenstoff-Verbindungen," 3rd ed., Voss, Hamburg and Leipzig (1910).

relationship between pyrimidine and purine (IV) is best expressed, however, by what may be called the ureide structure (pyrimidine A) as expressed by formula I. According to this system the numbering of pyrimidine positions is in agreement with that shown for purine in formula IV. An alternative system of numbering pyrimidine derivatives is used by Meyer and Jacobson in their well-known textbook.⁷



According to the Geneva nomenclature the four naturally occurring pyrimidines, formed by degradation of nucleic acids, are designated as follows:

NAME	PYRIMIDINE NOMENCLATURE	GENEVA NOMENCLATURE
Uracil	2,6-dioxypyrimidine	2,4-pyrimidinedione
Thymine	2,6-dioxo-5-methylpyrimidine	5-methyl-2,4-pyrimidinedione
Cytosine	2-oxy-6-aminopyrimidine	4-amino-2-pyrimidone
Methylcytosine	2-oxy-5-methyl-6-aminopyrimidine	4-amino-5-methyl-2-pyrimidone

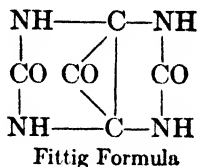
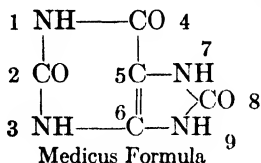
Purine is a condensed heterocycle consisting of a pyrimidine ring to which has been grafted a glyoxaline (imidazole) nucleus at positions 4 and 5, or 5 and 6 of the pyrimidine cycle. The name "purine" was first proposed by Emil Fischer in his classical paper on purine chemistry published in 1899.⁸ The accepted structure and nomenclature of purine

⁷ Meyer and Jacobson, "Lehrbuch der organischen Chemie," deGruyter, Leipzig (1920), Vol. II, part 3, p. 1172.

⁸ Fischer, *Ber.*, **32**, 435 (1899).

compounds are based on the old Medicus formula for uric acid.⁹ The Fittig formula¹⁰ for uric acid is of historical interest only, and so far as the author is aware no organic substance conforming to this constitution has thus far been synthesized.

URIC ACID



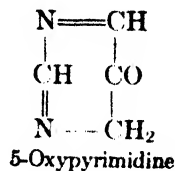
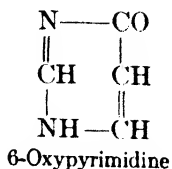
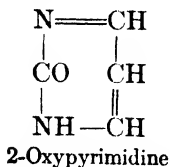
The utter confusion introduced by a change in the numbering of purine positions is illustrated by the numbering used in the compilation of ring systems published in Richter's "Lexikon."⁶ For purines this proposed numbering was not followed, however, and in the text of Richter's "Lexikon" the designation of positions in the purine cycle is in accord with that originally recommended by Emil Fischer.

CLASSIFICATION OF PYRIMIDINE COMPOUNDS

Pyrimidones

The pyrimidones may be grouped according to the following classification, namely, monoöxy-, dioxy-, trioxy-, and tetraoxypyrimidines. The monoöxy series includes the isomeric monoöxy pyrimidines, namely, 2-oxy-,¹¹ 5-oxy- and 6-oxypyrimidines,¹² and the corresponding saturated derivatives. The oxypyrimidines representative of these three types are expressed by the formulas which follow.

Monoöxypyrimidines



⁹ Medicus, *Ann.*, **175**, 243 (1875).

¹⁰ Fittig, "Grundriss der organische Chemie," Duncker and Humblot, Leipzig (1877).

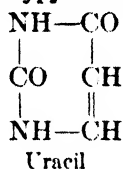
¹¹ Johnson and Joyce, *J. Am. Chem. Soc.*, **37**, 2151 (1915). See also "Acetylacetone," Stark, *Ann.*, **381**, 143 (1911); *Ber.*, **42**, 699, 708 (1909); Stark and Bögemann, *Ber.*, **43**, 1126 (1910); Evans, *J. prakt. Chem.*, [2] **46**, 352 (1892); [2] **48**, 489 (1893).

¹² Wheeler, *J. Biol. Chem.*, **3**, 285 (1907). See also for higher homologs Gabriel and Colman, *Ber.*, **32**, 1534 (1899); *Ber.*, **32**, 2930 (1899); Gabriel, *Ber.*, **37**, 3638 (1904).

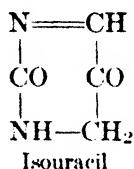
2-Oxypyrimidine,¹³ acetylacetone urea,¹⁴ anhydrodiacetone urea,¹⁵ and trimethylene urea¹⁶ are representatives of 2-oxypyrimidines. 6-Oxypyrimidine¹⁷ and a limited number of its derivatives¹⁸ are known. No 5-oxypyrimidines have been reported in the literature.

Uracil, thymine, and their reduced forms are the most important representatives of the 2,6-dioxypyrimidines. Isouracil¹⁹ and 4,6-dioxyhexahydropyrimidine²⁰ are members of the 2,5- and 4,6-dioxy series. No representatives of the 5,6-dioxypyrimidines have been described in the literature.

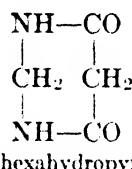
Dioxypyrimidines



Uracil



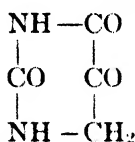
Isouracil



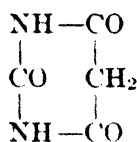
4,6-Dioxyhexahydropyrimidine

Barbituric acid²¹ and its several substituted derivatives, and isobarbituric acid,²² are the most important representatives of the trioxypyrimidine types. Alloxan²³ is the outstanding representative of the tetraoxy type of oxidized pyrimidines.

Trioxypyrimidines

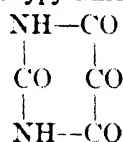


Isobarbituric acid



Barbituric acid

Tetraoxypyrimidines



Alloxan

¹³ Johnson and Joyce, *J. Am. Chem. Soc.*, **37**, 2151 (1915).

¹⁴ Stark, *Ann.*, **381**, 143 (1911); *Ber.*, **42**, 699, 708 (1909); Stark and Bögemann, *Ber.*, **43**, 1126 (1910); Evans, *J. prakt. Chem.*, [2] **46**, 352 (1892); [2] **48**, 489 (1893); Benary, Reiter, and Soenderop, *Ber.*, **50**, 69, 88 (1917); Hale and Vibrans, *J. Am. Chem. Soc.*, **40**, 1054, 1061 (1918).

¹⁵ Traube and Lorenz, *Ber.*, **32**, 3160 (1899); Traube, *Ber.*, **27**, 277 (1894).

¹⁶ Fischer and Koch, *Ann.*, **232**, 224 (1886); Tafel and Weinschenk, *Ber.*, **33**, 3386 (1900); Tafel and Reindl, *Ber.*, **34**, 3289 (1901); Franchimont and Friedmann, *Rec. trav. chim.*, **26**, 218 (1906); Curtius and Clemm, *J. prakt. Chem.*, [2] **62**, 197 (1900).

¹⁷ Wheeler, *J. Biol. Chem.*, **3**, 285 (1907).

¹⁸ Gabriel and Colman, *Ber.*, **32**, 1534, 2930 (1899); Gabriel, *Ber.*, **37**, 3638 (1904).

¹⁹ Tafel and Houseman, *Ber.*, **40**, 3748 (1907).

²⁰ Franchimont and Dubsky, *Rec. trav. chim.*, **36**, 84 (1916).

²¹ Baeyer, *Ann.*, **130**, 136 (1864); Finck, *Ann.*, **132**, 304 (1864); Baume, *Bull. soc. chim.*, [4] **31**, 146 (1922); Kopp, *Ber.*, **12**, 378 (1879); Conrad and Guthzeit, *Ber.*, **14**, 1643 (1881); **15**, 2844 (1882).

²² Behrend, *Ann.*, **229**, 39 (1885); Behrend and Roosen, *Ann.*, **251**, 239 (1889); Johnson and Jones, *Am. Chem. J.*, **40**, 545 (1908); Johns, *ibid.*, **45**, 83 (1911); Levene and La Forge, *Ber.*, **45**, 610, 619 (1912).

²³ For literature consult Beilstein's "Handbuch der organischen Chemie."

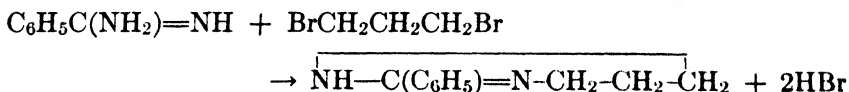
Reduced Pyrimidines

Relatively little is known of the chemistry of the reduced forms of pyrimidine. There are eight theoretically possible pyrimidine derivatives existing between pyrimidine, $C_4H_4N_2$,²⁴ and its completely reduced form hexahydropyrimidine, $C_4H_{10}N_2$.²⁵ Five members of this family of pyrimidines are isomeric modifications of dihydropyrimidine, $C_4H_6N_2$, and three are isomeric modifications of tetrahydropyrimidine, $C_4H_8N_2$. Several alkyl derivatives of pyrimidine have been prepared and the following may be mentioned: (1) monoalkylated: 2-methyl-1,3-diazine,²⁶ 4-methyl-1,3-diazine,²⁷ 5-methyl-1,3-diazine,²⁸ 5-ethyl-1,3-diazine,²⁹ and (2) dialkylated: 2,4-dimethyl-1,3-diazine,³⁰ 4,5-dimethyl-1,3-diazine,³¹ 4,6-dimethyl-1,3-diazine,³² 4-methyl-2-ethyl-1,3-diazine,³³ and 4-methyl-5-ethyl-1,3-diazine.³⁴ No representatives of the dihydropyrimidines have been described in the literature; and though no modification of tetrahydropyrimidine is known, alkyl derivatives of this series have been prepared, namely, 2-methyl-1,4,5,6-tetrahydro-1,3-diazine,³⁵ and the *cis* and *trans* isomers of 2,4,6-trimethyl-3,4,5,6-tetrahydro-1,3-diazine, $C_7H_{14}N_2$.³⁶

PYRIMIDINE SYNTHESIS

General Methods of Preparation

Since pyrimidines may be considered as cyclic amidines, many of the methods of preparation are based on a cyclization brought about by interaction of an acyclic amidine with a chain compound of three carbon atoms. 2-Phenyl-1,4,5,6-tetrahydropyrimidine, for example, may be obtained by condensing benzamidine with 1,3-dibromopropane.³⁷



²⁴ Gabriel, *Ber.*, **33**, 3666 (1900); Emery, *Ber.*, **34**, 4180 (1901); Gabriel and Colman, *Ber.*, **32**, 1537 (1899).

²⁵ Titherly and Branch, *J. Chem. Soc.*, **103**, 330 (1913); Branch, *J. Am. Chem. Soc.*, **38**, 2466 (1916). See also Bischoff and Reinfeld, *Ber.*, **36**, 35, (1903).

²⁶ Gabriel, *Ber.*, **37**, 3642 (1904).

²⁷ Gabriel and Colman, *Ber.*, **32**, 1535, 2921 (1899).

²⁸ Schlenker, *Ber.*, **34**, 2816 (1901); Gerngross, *Ber.*, **36**, 3396 (1905).

²⁹ Merckatz, *Ber.*, **52**, 869 (1919). See Kast, *Ber.*, **45**, 3124 (1912).

³⁰ Schmidt, *Ber.*, **35**, 1577 (1902).

³¹ Schlenker, *Ber.*, **34**, 2814 (1901).

³² Gabriel and Colman, *Ber.*, **32**, 1532 (1899); Angerstein, *Ber.*, **34**, 3957 (1901).

³³ Pinner, "Die Iminoäther u. ihre Derivate," Oppenheim, Berlin (1892), p. 224.

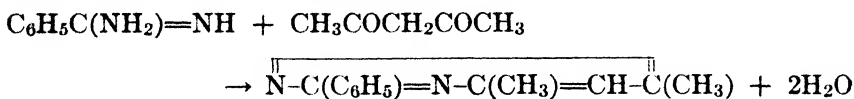
³⁴ Byk, *Ber.*, **36**, 1917 (1903).

³⁵ Hofmann, *Ber.*, **21**, 2336 (1888); Haga and Majima, *Ber.*, **36**, 334 (1903).

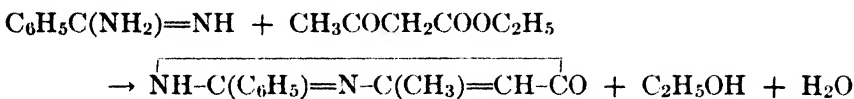
³⁶ Harries and Haga, *Ber.*, **32**, 1194, 1198 (1899).

³⁷ Pinner, *Ber.*, **26**, 2122 (1893).

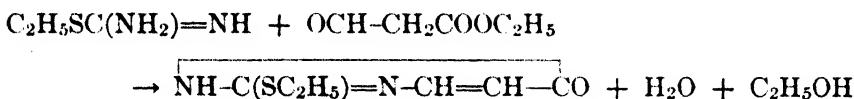
The application of this method of synthesis, however, is rather limited. A more fruitful method consists in condensing an amidine in alkaline solution with a β -diketone.³⁸ A similar condensation takes



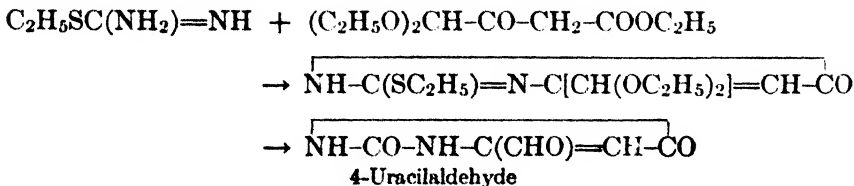
place readily between amidines and β -keto- or β -aldehydo-esters. In these cases keto forms of pyrimidine are formed.³⁹



Since isoureas and isothioureas (*pseudo* forms of urea and thiourea respectively) may be conceived as amidines, they can replace the latter in these condensation reactions. The pseudoalkylthioureas have been found to be very useful for synthesis of pyrimidines.⁴⁰ After cycliza-



tion, the mercapto group is easily removed by hydrolysis when an oxypyrimidine is formed. The methods of preparing pyrimidines, which are based on the application of pseudothiurea condensations, have been found to be extremely useful. They have led to pyrimidines which are otherwise almost inaccessible. By condensing pseudoethylthiourea with ethyl β -diethoxyacetoacetate, for example, a pyrimidine is formed which on hydrolysis in acid solution yields a pyrimidine containing an aldehyde group in the 4-position of the pyrimidine cycle. At present this is the only method available for preparing aldehydes of pyrimidines.⁴¹ The replacement of amidines by guanidine leads to derivatives



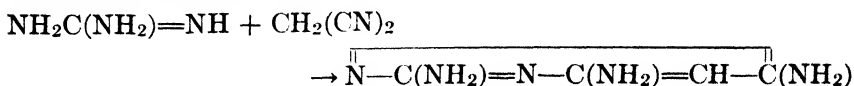
³⁸ Pinner, *Ber.*, **26**, 2124 (1893).

³⁹ Pinner, *Ber.*, **17**, 2519 (1884); **18**, 759, 2845 (1885).

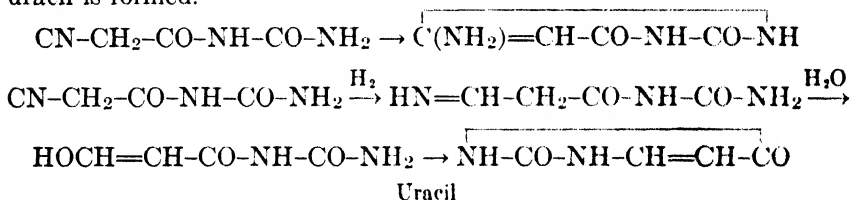
⁴⁰ Wheeler and Merriam, *Am. Chem. J.*, **29**, 484 (1903).

⁴¹ Johnson and Cletcher, *J. Am. Chem. Soc.*, **37**, 2144 (1915).

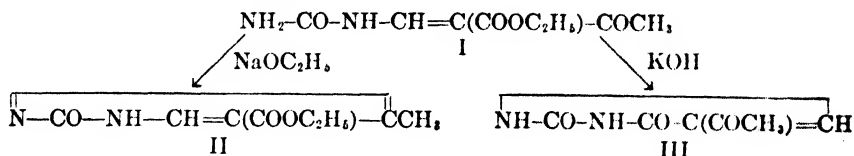
of 2-aminopyrimidines. In the presence of sodium ethylate, for example, guanidine and malononitrile add to each other with formation of 2,4,6-triaminopyrimidine.⁴²



The use of urea or thiourea in place of amidines leads directly to cyclic ureides, or pyrimidines containing oxygen or sulfur attached to the 2-position of the pyrimidine cycle. For instance, urea interacts with α -dichlorohydrin to form 2,5-dioxihexahydropyrimidine,⁴³ with ethyl acetoacetate in alkaline solution to form 4-methyluracil,⁴⁴ and with diethyl malonate to form barbituric acid.⁴⁵ Urea combines with cyanoacetic acid to form cyanoacetylurea. Cyclization of this urea is effected in two different ways. Under the influence of alkali, rearrangement to 4-aminouracil takes place,⁴⁶ and by catalytic hydrogenation uracil is formed.⁴⁷



The acyclic ureides are, in many instances, very useful compounds for synthesis since they offer the possibility of effecting ring closures in different directions, thereby leading to different types of pyrimidines. This is best illustrated by the following example. Ureido-methylene-ethylacetoacetate (I) reacts with sodium alcoholate to give the pyrimidine II, while under the influence of dilute potassium hydroxide solution followed by acidification, pyrimidine III, a ketone derivative of a 2,6-dioxypyrimidine, is formed.⁴⁸



⁴² Traube, *Ber.*, **37**, 4545 (1904).

⁴³ Turski and Kazmierczak, *Roczniki Chem.*, **13**, 375 (1933).

⁴⁴ Behrend, *Ann.*, **229**, 16 (1885).

⁴⁵ Gabriel and Colman, *Ber.*, **37**, 3657 (1904).

⁴⁶ Baum, *Ber.*, **41**, 538 (1908).

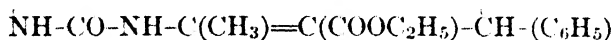
⁴⁷ Rupe, Metzger, and Volger, *Helv. Chim. Acta*, **8**, 850 (1925).

⁴⁸ Bergmann and Johnson, *Ber.*, **66**, 1494 (1933).

As has been shown above, cyclization can take place by way of addition of an amino or an imino group to the triple bond of a nitrile group. Under certain conditions they may also add to an ethylenic bond and thereby form the pyrimidine ring. Emil Fischer's synthesis of hydro-uracil⁴⁹ and Traube's⁵⁰ synthesis of 2-amino-4,4,6-trimethyl-4,5-dihydropyrimidine serve as examples of additions of this type.

The methods of synthesizing pyrimidines hitherto presented have dealt with an amidine or a urea derivative on the one hand, and on the other with an aliphatic chain compound containing three carbon atoms. That the presence of such an aliphatic chain construction is not necessary for the formation of the pyrimidine ring is shown in the reactions discussed below.

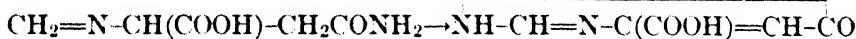
Urea, benzaldehyde, and ethyl acetoacetate interact to give 2-oxy-4-methyl-5-carbethoxy-6-phenyl-1,2,3,6-tetrahydropyrimidine.⁵¹ One



molecule of urea and two of phenylacetaldehyde combine to form 2-oxy-5-phenyl-6-benzyl-1,2,3,6-tetrahydropyrimidine.⁵²

The presence of an amidine or a urea derivative is not necessary for the formation of a pyrimidine ring. For example, 2-methyl-3,4,5,6-tetrahydropyrimidine is formed by interaction of acetic acid and 1,3-diaminopropane.⁵³

Of biochemical interest is the formation of a pyrimidine compound from aspartic acid. The amide of this amino acid reacts with formaldehyde to give a methylene derivative, which by oxidation is transformed into 6-oxy-4-pyrimidinecarboxylic acid.⁵⁴



For the formation of a pyrimidine cycle, neither the presence of an amidine nor urea nor an aliphatic chain of three carbon atoms is necessary. This was established by the characteristic polymerization of nitriles leading to the formation of the so-called cyanalkines.⁵⁵ These

⁴⁹ Fischer and Roeder, *Ber.*, **34**, 3759 (1901).

⁵⁰ Traube and Schwarz, *Ber.*, **32**, 3163 (1899).

⁵¹ Biginelli, *Ber.*, **24**, 1317 (1891); Hinkel and Hey, *Rec. trav. chim.*, **48**, 1283 (1929); Johnson, Folkers, and Harwood, *J. Am. Chem. Soc.*, **54**, 3751 (1932); Folkers and Johnson, *ibid.*, **55**, 3784 (1933).

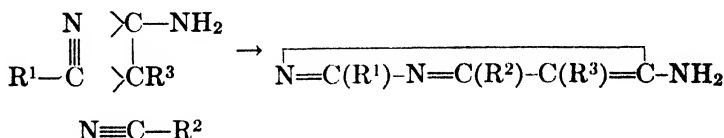
⁵² Folkers and Johnson, *ibid.*, **55**, 3361 (1933).

⁵³ Haga and Majima, *Ber.*, **36**, 334 (1903); Hofmann, *Ber.*, **21**, 2336 (1888).

⁵⁴ Cherbuliez and Stavritsch, *Helv. Chim. Acta*, **5**, 267 (1922).

⁵⁵ Wache, *J. prakt. Chem.*, **39**, 247, 256 (1889); v. Meyer, *ibid.*, **37**, 397 (1888); Herfeldt, *ibid.*, **53**, 246 (1896).

condensations also take place between nitriles of different structures. For the formation of the pyrimidine ring, however, it is necessary that one of the nitriles taking part in the reaction be primary, because the reaction probably proceeds as follows:

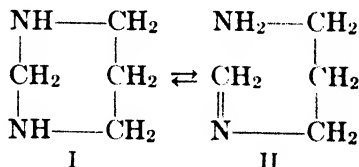


Benzonitrile alone does not polymerize to give a pyrimidine, but two molecules of this nitrile and one of phenylacetoneitrile, for example, interact to form such a cycle. Finally, pyrimidines are obtained by the degradation of purines. The complete hydrolysis of adenine, for example, leads to the formation of 6-aminopyrimidine,⁵⁶ and the oxidation of uric acid with chlorine produces alloxan.⁵⁷

Special Methods of Preparation

Preparation of Pyrimidine and Its Homologs. Pyrimidine may be synthesized in either of the following ways: (1) Barbituric acid is first treated with phosphorus oxychloride to form 2,4,6-trichloropyrimidine, which is then reduced to pyrimidine by means of zinc dust.⁵⁸ (2) Homologs of pyrimidine are oxidized to the corresponding carboxylic acids which are then converted into pyrimidine by application of heat, bringing about decarboxylation.⁵⁹

Hydropyrimidines result by interaction of 2,4-diaminopentanes with acetic acid. Two products are formed (*cis* and *trans*), depending on whether the reaction is carried out with the *meso* or racemic modification of the amine.⁶⁰ Hexahydropyrimidine (I) is obtained by interaction of formaldehyde and 1,3-diaminopropane. It has not been possible to



⁵⁶ Burian, *Ergeb. Physiol. (Asher Spiro)*, **5**, 794 (1906).

⁵⁷ Biltz and Heyn, *Ann.*, **413**, 60 (1917).

⁵⁸ Gabriel, *Ber.*, **33**, 3666 (1900); Büttner, *Ber.*, **36**, 2227 (1903); Gabriel and Colman, *Ber.*, **37**, 3657 (1904).

⁵⁹ Gabriel and Colman, *Ber.*, **32**, 1537 (1899).

⁶⁰ Harries and Haga, *Ber.*, **32**, 1191 (1899).

isolate it, however, since it changes quickly into its tautomeric form, α -methyleneamino- γ -aminopropane (II). In solution, both forms are in equilibrium. On addition of benzoyl chloride to the solution, N, N'-dibenzoylhexahydropyrimidine is formed and can be isolated.⁶¹

Pyrimidinecarboxylic acids are obtained by oxidation of homologs of pyrimidine with potassium permanganate.⁶² Quinazoline under similar conditions yields 4,5-pyrimidinedicarboxylic acid which on heating loses carbon dioxide to give 5-pyrimidinecarboxylic acid.⁶³

Pyrimidine halides can be prepared by the direct action of a halogen on pyrimidines.⁶⁴ Thereby substitution takes place in the 5-position of the cycle. 2-Phenyl-5-bromopyrimidine has been obtained by decarboxylation of the corresponding 4-carboxylic acid. The latter is formed also by condensation of mucobromic acid with benzamidine.⁶⁵

The replacement of hydroxyl groups in oxypyrimidines by chlorine atoms by treating the oxypyrimidine with phosphorus oxychloride and phosphorus pentachloride is an extremely useful method for the preparation of chloropyrimidines. As an example, the synthesis of 2,4,6-trichloropyrimidine from barbituric acid has already been referred to in this chapter. This reaction can be applied to all oxypyrimidines and their homologs. 4-Methyluracil on chlorination gives 2,6-dichloro-4-methylpyrimidine and alloxan yields 2,4,5,6-tetrachloropyrimidine.⁶⁶ Iodine cannot be introduced directly into the pyrimidine nucleus. However, it has been possible to replace chlorine atoms occupying the 4- and 6-positions of the pyrimidine cycle by iodine.⁶⁷

Aminopyrimidines. Most of the important mono-, di-, and poly-amino derivatives of pyrimidine have been prepared by the action of alcoholic ammonia on the halogen derivatives of pyrimidine and its homologs. The advantage of this method is that the replacement of one or more chlorine atoms in pyrimidine polyhalides by the amino group depends on the temperature of reaction.

By treating 2,4,6-trichloropyrimidine with alcoholic ammonia below 100° one chlorine atom only is replaced by an amino group. The reaction product consists of a mixture of pyrimidines I and II which can be separated easily by crystallization. At 160° the trichloro compound as well as pyrimidines I and II yield the pyrimidine III, and at temper-

⁶¹ Titherley and Branch, *J. Chem. Soc.*, **103**, 330 (1913); Branch, *J. Am. Chem. Soc.* **38**, 2466 (1916).

⁶² Schlenker, *Ber.*, **34**, 2815 (1901); Angerstein, *Ber.*, **34**, 3957 (1901).

⁶³ Gabriel and Colman, *Ber.*, **37**, 3047 (1904).

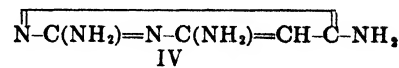
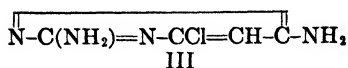
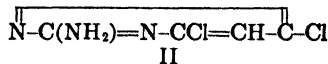
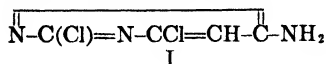
⁶⁴ Johnson and Johns, *Am. Chem. J.* **34**, 185 (1905).

⁶⁵ Kunkell and Zumbusch, *Ber.*, **35**, 3165 (1902).

⁶⁶ Ciamician and Magnaghi, *Ber.*, **18**, 3445 (1885); Emery, *Ber.*, **34**, 4178 (1901).

⁶⁷ Gabriel and Colman, *Ber.*, **32**, 2931 (1899).

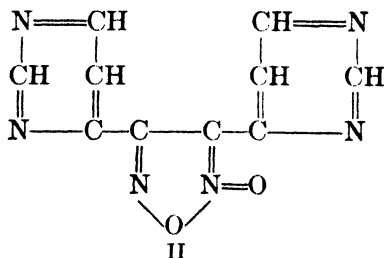
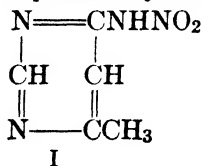
atures above 300° the last of the chlorine atoms is removed and 2,4,6-triaminopyrimidine (IV) is formed.⁶⁸



In all three of the pyrimidines I, II, and III the chlorine atoms can be replaced by hydrogen. When reduced with zinc dust the triaminopyrimidine IV is converted into 2-aminopyrimidine⁶⁹ with loss of ammonia. In order to obtain 6-aminopyrimidine from III it is necessary to reduce this with hydrogen iodide and phosphorus when 4-iodo-6-aminopyrimidine is formed. On reduction with zinc dust this iodo compound is converted into 6-aminopyrimidine. In a similar manner pyrimidine III can be reduced to 2,6-diaminopyrimidine.⁷⁰

The chlorine atom in the 5-position of the pyrimidine nucleus is not easily replaced directly by an amino group. In order to prepare 5-aminopyrimidines the pyrimidine has first to be subjected to nitration whereby the nitro group generally goes into the 5-position. The nitro compound can then be reduced easily to the corresponding amino compound.⁷¹ 2,4,6-Triaminopyrimidine reacts with nitrous acid to give 5-nitroso-2,4,6-triaminopyrimidine, which can be reduced to 2,4,5,6-tetraaminopyrimidine.⁷²

Nitropyrimidines can be prepared by direct nitration. The nitro group, however, does not always enter the 5-position. 4-Methyl-6-aminopyrimidine, for example, reacts with nitric acid to give the pyrimidine⁷³ represented by Formula I, while 4-methylpyrimidine yields the product expressed by Formula II.⁷⁴



⁶⁸ Gabriel and Colman, *Ber.*, **37**, 3657 (1904).

⁶⁹ Büttner, *Ber.*, **36**, 2229, 2232 (1903).

⁷⁰ Büttner, *Ber.*, **36**, 2233 (1903).

⁷¹ Isay, *Ber.*, **39**, 255 (1906).

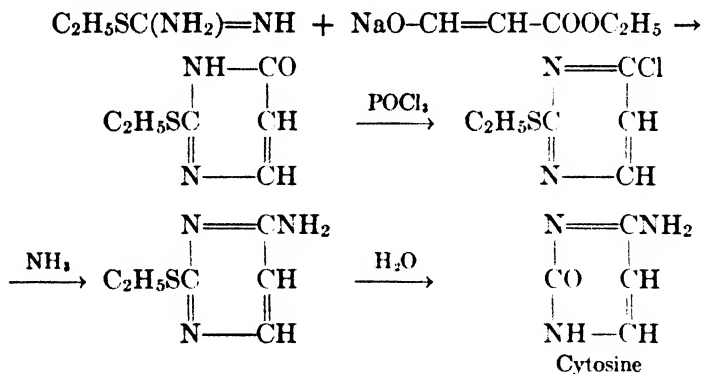
⁷² Traube, *Ber.*, **37**, 4546 (1904).

⁷³ Gabriel and Colman, *Ber.*, **34**, 1241 (1901).

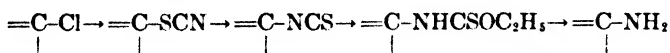
⁷⁴ Gabriel and Colman, *Ber.*, **35**, 1573 (1902).

Mercaptopyrimidines. Pyrimidines containing a mercapto group in the 2-position have been prepared in large numbers. They are formed by condensing pseudoalkylthioureas with β -ketonic esters and β -diketones (see above). A profitable way to prepare them, especially those having the mercapto group in the 4- or 6-positions, is the replacement of the chlorine atoms of a chloropyrimidine by the sulfhydryl group by action of potassium hydrogen sulfide.⁷⁵ Direct alkylation of the resulting thiopyrimidines leads to the corresponding mercapto derivatives.⁷⁶

Under the action of hydrobromic or hydrochloric acid the mercapto group can be replaced by hydroxyl and an oxypyrimidine formed. The mercapto group can be replaced also by an amino group, in some cases, by treatment with ammonia. Phosphorus oxychloride and phosphorus pentachloride have no destructive influence on the mercapto group. This property makes the 2-mercaptopyrimidines important and useful intermediary products, a fact which is best illustrated by the following synthesis of cytosine.⁷⁷



Thiocyano derivatives of pyrimidines may be obtained by the interaction of chloropyrimidines with potassium thiocyanate. The resulting thiocyanates can be rearranged to isothiocyanates and the latter converted to thiourethanes and amines.⁷⁸



Alkoxyprymidines can be prepared from the corresponding halogen derivatives by the action of sodium alcoholates. Similarly to the

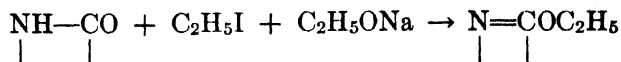
⁷⁵ Wheeler and Liddle, *Am. Chem. J.*, **40**, 557 (1908).

⁷⁶ Traube, *Ann.*, **331**, 80 (1904).

⁷⁷ Wheeler and Johnson, *Am. Chem. J.*, **29**, 492 (1903).

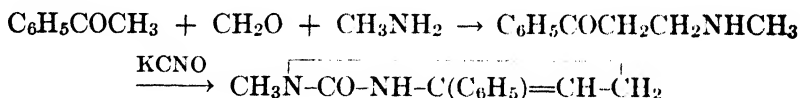
⁷⁸ Johnson and Chi, *J. Am. Chem. Soc.*, **52**, 1584 (1930).

formation of aminopyrimidines from 2,4,6-trichloropyrimidine, mono-, di-, and trialkoxy derivatives can be obtained if the temperature of the reaction between the sodium alcoholate and the dichloropyrimidine is properly controlled.⁷⁹ It is also possible to prepare alkoxyprymidines by direct alkylation of oxyprymidines in the form of their sodium salts.⁸⁰



Since the alkoxyprymidines tend to undergo molecular rearrangements into the *lactam* modifications they can be used as intermediary products for the formation of nitrogen alkylation derivatives of pyrimidines. 2,6-Dimethoxyprymidine, for example, rearranges into 1,3-dimethyluracil.⁸¹ The *lactim* form on treatment with methyl iodide yields 2-oxy-3-methyl-6-methoxyprymidine. The latter can be transformed into 3-methyluracil by hydrolysis. This property of 2,6-dimethoxyprymidine has been employed in the synthesis of 3-glucosidouracil.⁸²

Monoöxyprymidines. 2-Oxyprymidine is easily prepared from 2-ethylmercapto-6-oxyprymidine.⁸³ 4,6-Dimethyl-2-oxyprymidine (acetylacetoneurea) is readily available by condensation of acetylacetone with urea.⁸⁴ Derivatives of 2-oxy-1,6-dihydropyrimidine (desoxouracil) can be obtained by application of the Biginelli reaction,⁸⁵ by reduction of 2-oxyprymidines,⁸⁶ and also by means of the following reactions.⁸⁷



2-Oxytetrahydropyrimidine is formed by heating trimethylenediamine with ethyl carbonate.⁸⁸

4-Oxyprymidines are known in larger numbers than the types mentioned above. They are obtained by condensation of amidines with β -ketonic esters and by replacement of amino groups in pyrimidines by

⁷⁹ Büttner, *Ber.*, **36**, 2234 (1903).

⁸⁰ Johnson and Moran, *J. Am. Chem. Soc.*, **37**, 2595 (1915).

⁸¹ Hilbert and Johnson, *ibid.*, **52**, 2001 (1930).

⁸² Hilbert and Johnson, *ibid.*, **52**, 4489 (1930).

⁸³ Johnson and Joyce, *ibid.*, **37**, 2150 (1915).

⁸⁴ Evans, *J. prakt. Chem.*, **48**, 492 (1893).

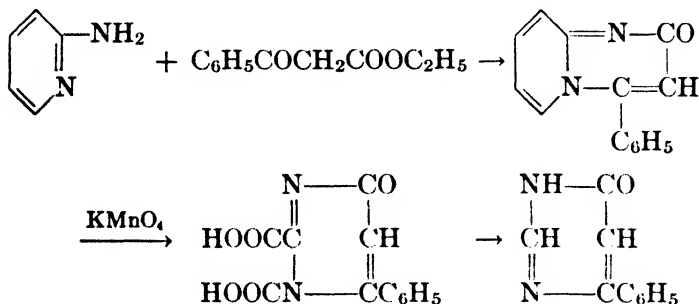
⁸⁵ Folkers and Johnson, *J. Am. Chem. Soc.*, **55**, 3361 (1933).

⁸⁶ Bergmann and Johnson, *Ber.*, **66**, 1492 (1933).

⁸⁷ Mannich and Heilner, *Ber.*, **55**, 365 (1922).

⁸⁸ Fischer and Köch, *Ann.*, **232**, 224 (1886).

treatment with nitrous acid.⁸⁹ 4-Oxypyrimidine (pyrimidone) is formed by the action of hydrogen iodide and red phosphorus on 2,4-dichloropyrimidine.⁹⁰ 6-Phenyl-4-oxypyrimidine has been obtained by the following series of reactions.⁹¹



A 6-aldehyde derivative of a 4-oxypyrimidine has been described.⁹² **Monoaminomonoöxypyrimidines.** These can be prepared by application of standard methods of synthesis already discussed. Acetamidine and ethyl cyanoacetate interact, for example, to form 2-methyl-4-oxy-6-aminopyrimidine.⁹³ The condensation of guanidine and β -aldehyde or β -ketoesters leads to the formation of 2-amino-4-oxypyrimidines. The synthesis of isocytosine illustrates such a reaction.⁹⁴ The preparation of 2-oxy-6-aminopyrimidine (cytosine) has already been referred to. Another way to prepare cytosine avoids the use of intermediary sulfur compounds and uses uracil as the starting point. The latter is converted to 2,6-dichloropyrimidine which upon treatment with ammonia yields a mixture of aminochloropyrimidines. The halogens are then replaced by methoxy groups by treatment with sodium methoxide. Isomers are obtained which are easily separated. On hydrolysis the 2-methoxy-6-aminopyrimidine yields cytosine, and the 2-amino-6-methoxypyrimidine gives isocytosine.⁹⁵ For the preparation of other cytosine derivatives consult the literature.⁹⁶

Monoöxydiaminopyrimidines. 2-Oxy-5,6-diamino- and 6-oxy-2,5-diaminopyrimidine, 5-aminocytosine,⁹⁷ and 5-aminoisocytosine⁹⁸ are

⁸⁹ v. Meyer, *J. prakt. Chem.*, [2] **37**, 409 (1888).

⁹⁰ Wheeler, *J. Biol. Chem.*, **3**, 288 (1907).

⁹¹ Seide, *Ber.*, **58**, 352 (1925).

⁹² Johnson and Mikeska, *J. Am. Chem. Soc.*, **42**, 2349 (1920).

⁹³ Traube, *Ger. pat.* 135, 371; *Chem. Zentr.*, (II), 1229 (1902).

⁹⁴ Wheeler and Johnson, *Am. Chem. J.*, **29**, 501 (1903).

⁹⁵ Hilbert and Johnson, *J. Am. Chem. Soc.*, **52**, 1154 (1930).

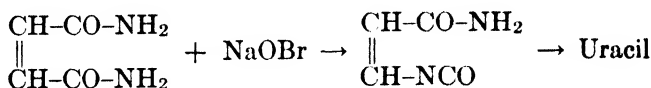
⁹⁶ Wheeler and Johnson, *Am. Chem. J.*, **31** 591 (1904); Johns, *ibid.*, **40**, 348 (1908); Johnson and Clapp, *J. Biol. Chem.*, **5**, 49, 62 (1908).

⁹⁷ Wheeler and Johnson, *Am. Chem. J.*, **31**, 591 (1904).

⁹⁸ Johnson and Johns, *ibid.*, **34**, 559 (1905).

prepared by reducing the corresponding 5-nitropyrimidines. The condensation of guanidine with ethyl cyanoacetate leads to 6-oxy-2,4-diaminopyrimidine.⁹⁹ 2-Methylmercapto-4,6-diaminopyrimidine is converted into 2-oxy-4,6-diaminopyrimidine by hydrolysis with acids.¹⁰⁰

Dioxypyrimidines. For the preparation of 2,6-dioxypyrimidine (uracil) several methods are available, but most of them are only of historical or theoretical interest. Fischer prepared the compound from 5-bromodihydrouracil.¹⁰¹ It can also be obtained by a series of reactions from 2,6-dimethoxy-4-chloropyrimidine.¹⁰² Another method is based on a partial Hofmann reaction (p. 694) applied to maleic diamide.¹⁰³



Of the greatest practical value is the synthesis of uracil by interaction of urea and malic acid in fuming sulfuric acid.¹⁰⁴ In this reaction malic acid is changed by the action of strong sulfuric acid into formylacetic acid which combines with urea to form uracil.

Homologs of uracil have been synthesized by methods which are analogous to those used for the preparation of uracil. Thymine (5-methyluracil) can be prepared (1) from urea and methacrylic acid,¹⁰⁵ (2) from pseudoethylthiourea and ethyl formylpropionate,¹⁰⁶ (3) from methylecyanoacetylurea on catalytic reduction,¹⁰⁷ and (4) from 5-methyl-2,4,6-trichloropyrimidine.¹⁰⁸

The classical method of Behrend is available for the preparation of 4-methyluracil.¹⁰⁹ Curiously enough this method cannot be applied with success to substituted ureas or esters of alkylated ethyl acetoacetate. Such compounds can be obtained indirectly through condensations with guanidine.¹¹⁰ N-Methyl homologs of uracil, for example, 1,3-dimethyluracil, can be prepared by methylation of uracil with diazomethane¹¹¹ or by the rearrangement of 2,6-dimethoxypyrim-

⁹⁹ Traube, *Ber.*, **33**, 1375, 3035 (1900).

¹⁰⁰ Wheeler and Jamieson, *Am. Chem. J.*, **32**, 349 (1904).

¹⁰¹ Fischer and Roeder, *Ber.*, **34**, 3761 (1901).

¹⁰² Gabriel and Colman, *Ber.*, **36**, 3379 (1903).

¹⁰³ Rinkes, *Rec. trav. chim.*, **46**, 268 (1927).

¹⁰⁴ Davidson and Baudisch, *J. Am. Chem. Soc.*, **48**, 2379 (1926).

¹⁰⁵ Fischer and Roeder, *Ber.*, **34**, 3751, 3758 (1901).

¹⁰⁶ Wheeler and Merriam, *Am. Chem. J.*, **29**, 478 (1903).

¹⁰⁷ Bergmann and Johnson, *J. Am. Chem. Soc.*, **55**, 1733 (1933).

¹⁰⁸ Gerngross, *Ber.*, **38**, 3408 (1905).

¹⁰⁹ Behrend, *Ann.*, **229**, 16 (1885).

¹¹⁰ Byk, *Ber.*, **36**, 1915 (1903).

¹¹¹ Johnson, Hill and Case, *Proc. Natl. Acad. Sci., U. S.*, **8**, 44 (1922).

idine.¹¹² Several derivatives of uracil have been prepared which contain oxygen in the side chain, namely, 5-methyluracil-4-methanol and derivatives,¹¹³ and also 4-methyluracil-5-methanol by the action of formaldehyde on 4-methyluracil.¹¹⁴

Amino Derivatives of Dioxypyrimidines. A general method for the preparation of 5-amino-2,4-dioxypyrimidines (5-aminouracils) consists in reducing the corresponding nitro compound.¹¹⁵ 5-Bromouracil¹¹⁶ and 5-nitrouracil¹¹⁷ are both easily accessible pyrimidines, but replacement of a halogen in position-5 by the action of ammonia is not a practical procedure. Of the dioxydiaminopyrimidines, 4,6-dioxy-2,5-diaminopyrimidine (divicine) and 2,6-dioxy-4,5-diaminopyrimidine have been prepared by the reduction of the corresponding 5-isonitrosopyrimidines.¹¹⁸

Hydrogenated Dioxypyrimidines. 2,6-Dioxy-4,5-dihydropyrimidine (dihydrouracil) can be prepared by the catalytic reduction of uracil, by condensation of urea with acrylic acid, by cyclization of β -ureidoacrylic acid,¹¹⁹ and also by interaction of the diamide of succinic acid and sodium hypobromite.¹²⁰ The electrolytic reduction of veronal (5,5-diethylbarbituric acid) leads to a derivative of an isomer of hydrouracil, namely, 2-desoxyveronal.¹²¹

Trioxypyrimidines. Barbituric acid is the outstanding representative of this series, and has already been referred to above. It may be obtained by heating urea with malonic acid in the presence of phosphorus oxychloride.¹²² Owing to the pharmacological interest of derivatives of barbituric acid a large number of homologs have been prepared. 5,5-Dimethylbarbituric acid is obtained by interaction of the silver salt of barbituric acid and methyl iodide.¹²³ This method, however, cannot be applied successfully for introduction of alkyl groups higher than methyl. Approaches to the higher homologs exist in the condensation of mono- and dialkylmalonyldichlorides, mono- and dialkylmalonic esters, mono- and dialkylethanoacetic esters, and mono- and dialkylmalononitriles with urea, thiourea, or guanidine. Thereby,

¹¹² Hilbert and Johnson, *J. Am. Chem. Soc.*, **52**, 2001 (1930).

¹¹³ Johnson and Chernoff, *ibid.*, **35**, 585 (1913).

¹¹⁴ Kircher, *Ann.*, **385**, 293 (1921); Schmedes, *Ann.*, **441**, 192 (1925).

¹¹⁵ Behrend, *Ann.*, **240**, 23 (1887); Köhler, *Ann.*, **236**, 50 (1886). See Michael, *J. prakt. Chem.*, [2] **35**, 456 (1887).

¹¹⁶ Wheeler and Merriam, *Am. Chem. J.*, **29**, 486 (1903).

¹¹⁷ Behrend, *Ann.*, **229**, 35 (1885).

¹¹⁸ Traube, *Ber.*, **26**, 2555 (1893); **33**, 1382, 3044 (1900).

¹¹⁹ Lengfeld and Stieglitz, *Am. Chem. J.*, **15**, 516 (1893).

¹²⁰ Weidel and Roithner, *Monatsh.*, **17**, 182 (1896).

¹²¹ Tafel and Thompson, *Ber.*, **40**, 4489 (1907).

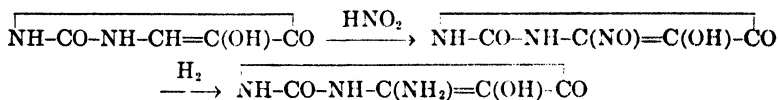
¹²² Grimaux, *Bull. soc. chim.* [3] **31**, 146 (1879).

¹²³ Conrad and Guthzeit, *Ber.*, **14**, 1643 (1881).

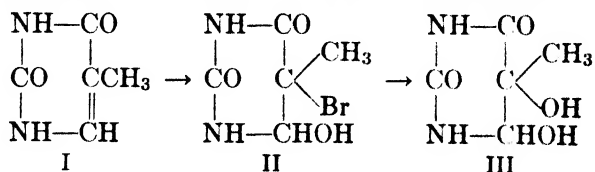
5-mono- or dialkylbarbituric acids, or the corresponding thio- and imino-derivatives are obtained by condensation in presence of sodium alkoxides. The latter on hydrolysis give the corresponding barbituric acids. N-Alkyl and N-aryl barbituric acids have also been prepared.¹²⁴ During recent years increased attention has been given to the chemical synthesis and pharmacological study of thiobarbiturates.

5-Bromo-, 5-nitro- (dilituric acid), and 5-isonitroso- (violuric acid) barbituric acids are formed by direct action of bromine, nitric acid, and nitrous acid, respectively, on barbituric acid.¹²⁵ Isobarbituric acid is obtained as a secondary product by the reduction of 5-nitrouracil.¹²⁶

Amino Derivatives of Trioxypyrimidines. Of the many ways which lead to 2,4,6-trioxo-5-aminopyrimidine (uramil), only three will be mentioned: (1) reduction of dilituric and violuric acid,¹²⁷ (2) interaction of alloxan and ammonium sulfite,¹²⁸ and (3) action of ammonia on dialuric acid.¹²⁹ 2,5,6-Trioxo-4-aminopyrimidine (isouramil) can be prepared from isobarbituric acid.¹³⁰



Tetraoxypyrimidines. The outstanding representative of this series is alloxan, which is obtained by oxidation of uric acid. The reduction of alloxan leads to dialuric acid.¹³¹ The isomeric isodialuric acid results from the oxidation of isobarbituric acid with bromine water.¹³² A 5-methyl derivative of 2,4,5,6-tetraoxypyrimidine is the thymine glycol,¹³³ which is formed as follows: Thymine (I) is converted into 4-hydroxy-5-bromohydrothymine (II) by treating it with bromine in aqueous solution. This bromo compound is then allowed to interact



¹²⁴ Hepner and Frankenberg, *Ber.*, **65**, 123 (1932).

¹²⁵ Baeyer, *Ann.*, **127**, 220, 223 (1863); Ceresole, *Ber.*, **16**, 1133 (1883).

¹²⁶ Behrend and Grünwald, *Ann.*, **309**, 256 (1899).

¹²⁷ Baeyer, *Ann.*, **127**, 220, 223 (1863).

¹²⁸ Fischer and Clemm, *Ber.*, **30**, 3091 (1897).

¹²⁹ Biltz and Damm, *Ber.*, **46**, 3668 (1913).

¹³⁰ Bogert and Davidson, *Proc. Natl. Acad. Sci. U. S.*, **18**, 490 (1932).

¹³¹ Wöhler and Liebig, *Ann.*, **26**, 276 (1838); Biltz and Damm, *Ber.*, **46**, 3663 (1913).

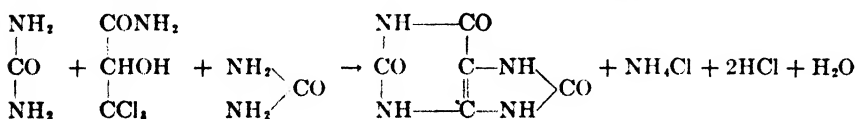
¹³² Behrend and Roosen, *Ber.*, **21**, 999 (1888); Johnson and Jones, *Am. Chem. J.*, **40**, 546 (1908).

¹³³ Baudisch and Davidson, *J. Biol. Chem.*, **64**, 233 (1925).

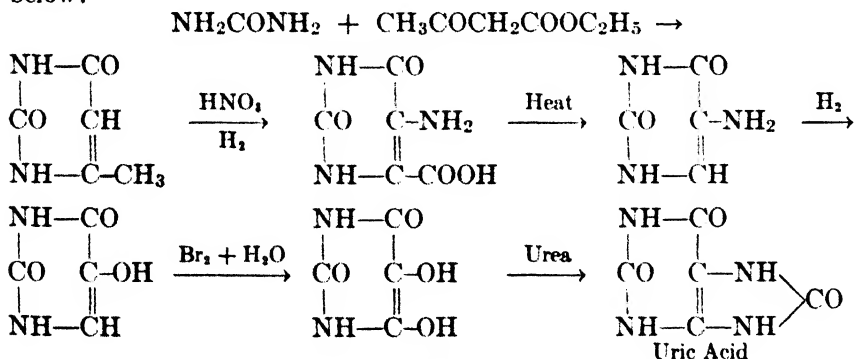
with silver oxide whereby the bromine is removed and thymine glycol (III) is formed.

PURINE SYNTHESIS

The first synthesis of a purine was accomplished by Horbaczewski¹³⁴ in 1882 when he reported the formation of uric acid by the fusion of glycocoll with urea. Several years later¹³⁵ he replaced glycocoll with the amide of trichlorolactic acid and reported the formation of uric acid in improved yields. It is interesting to note that Robert Behrend later repeated this pioneer work of Horbaczewski and was unable to produce uric acid by the fusion of urea with glycocoll. However, when trichlorolactamide was employed uric acid was obtained.¹³⁶



This method of synthesis revealed very little concerning the exact structure of the uric acid molecule, and it remained for Behrend and Roosen¹³⁷ to present the first synthesis of uric acid which showed the constitution of this purine. These investigators condensed urea with ethyl acetoacetate to form the pyrimidine 4-methyluracil. Upon treatment of this compound with nitric acid, nitration and oxidation took place with the formation of 5-nitro-4-uracilcarboxylic acid which readily lost carbon dioxide upon heating to form 5-nitrouracil. Reduction of the latter with tin and hydrochloric acid gave a mixture of 5-amino- and 5-hydroxyuracil. The latter compound was then oxidized with bromine water to isodialuric acid which reacts with urea in the presence of sulfuric acid to form uric acid. These changes are expressed below:



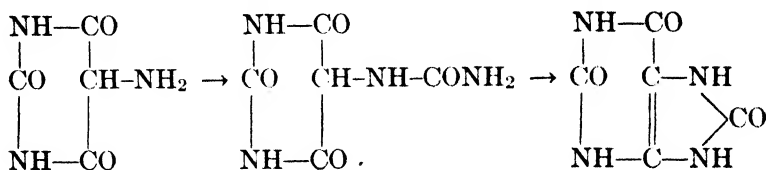
¹³⁴ Horbaczewski, *Monatsh.*, **3**, 792 (1882); **6**, 356 (1885).

¹³⁵ Horbaczewski, *ibid.*, **8**, 201 (1887).

¹³⁶ Behrend, *Ann.*, **441**, 215 (1925).

More recently this method of synthesis has been employed for the preparation of several methyl derivatives of uric acid.¹³⁸ Owing to the present availability of uracil, 5-nitrouracil can be more readily prepared from this and the uric acid synthesis accordingly simplified.

The next important method of synthesizing uric acid was described by Fischer and Ach,¹³⁹ who used as their starting material 5-amino-barbituric acid from which was prepared pseudouric acid. Upon fusion with oxalic acid, or by heating with hydrochloric acid, cyclization of the latter pyrimidine is brought about with formation of uric acid as follows:



Methylated uramils may also be employed in this synthesis¹⁴⁰ and methylpseudouric acids obtained, or, as Biltz has shown, pseudouric acid may be methylated directly.¹⁴¹ The resulting alkyl pseudouric acids upon ring closure yield the corresponding alkyl uric acids. The following purines of the uric acid type were thus prepared: 1-methyluric acid, 7-methyluric acid, 1,3-dimethyluric acid, 1,7-dimethyluric acid, and 1,3,7-trimethyluric acid. Since these methylated uric acids can be utilized indirectly for the preparation of purine bases (xanthine derivatives), the method is possible of wide application.

A fruitful method for the preparation of purine derivatives from uric acid and the alkylated uric acids was discovered in the formation of halogen derivatives of purine by interaction of uric acids with the halides of phosphorus.¹⁴² Owing to their pronounced reactivity, the halogen atoms of the purine undergo reaction with a variety of reagents such as alkali, ammonia, reducing agents, and sodium alkoxides. This has made possible the synthesis and interconversion of all the more important purine bases. For example, when uric acid is heated with phosphorus oxychloride, chlorine first enters the 2- and 6-positions of the purine ring.¹⁴³

¹³⁷ Behrend and Roosen, *Ann.*, **251**, 235 (1889).

¹³⁸ v. Loeben, *Ann.*, **298**, 181 (1897); Prüsse, *Ann.*, **441**, 203 (1925).

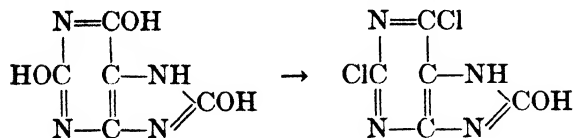
¹³⁹ Fischer and Ach, *Ber.*, **28**, 2473 (1895); Fischer, *Ber.*, **30**, 559 (1897).

¹⁴⁰ Fischer and Clemm, *Ber.*, **30**, 3089 (1897); Fischer, *Ber.*, **30**, 563 (1897); Fischer and Ach, *Ber.*, **28**, 2475 (1895); Techow, *Ber.*, **27**, 3087 (1894); Biltz and Damm, *Ber.*, **46**, 3670 (1913).

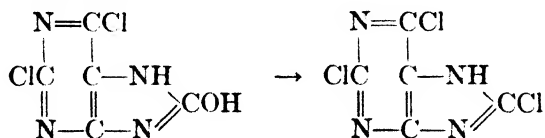
¹⁴¹ Biltz and Heyn, *Ann.*, **423**, 190 (1921).

¹⁴² Fischer, "Untersuchungen in der Puringruppe," Springer, Berlin (1907).

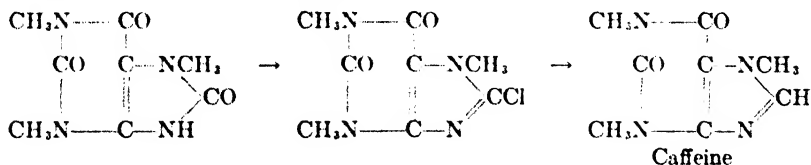
¹⁴³ Fischer and Ach, *Ber.*, **30**, 2208 (1897).



At higher temperatures, using a large excess of the phosphorus halide, chlorine substitutes in the 8-position.¹⁴⁴



The chlorine in the 6-position is the most reactive; the 8-position contains the least reactive halogen atom. With these facts in mind, Fischer, starting with 2,6,8-trichloropurine, successfully prepared purine,¹⁴⁵ xanthine, adenine, guanine, and hypoxanthine.¹⁴⁶ The action of the phosphorus halides upon alkylated uric acids proceeds in a manner quite different from that in the case of uric acid. This is due largely to the inability of such compounds to act in the enolic form (lactim) because of the substitution of the mobile hydrogen atom of $-\text{CO}-\text{NH}-$ groups by the alkyl radicals. Thus 1,3,7-trimethyluric acid when heated with a mixture of phosphorus oxychloride and phosphorus pentachloride is converted into 8-chlorocaffeine,¹⁴⁷ which is easily reduced to caffeine. In like manner, 1,3-dimethyluric acid may be converted into 8-chloro-



theophylline,¹⁴⁸ and the latter reduced to theophylline or 1,3-dimethylxanthine.

Later Fischer¹⁴⁹ employed phosphorus oxychloride alone, and by its action he was able to convert 3-methyl-, 3,7-dimethyl-, and 1,7-dimethyluric acids, respectively, into the corresponding 8-chloropurine derivatives. These halogenated purines are all easily reduced to the corresponding xanthine bases.

¹⁴⁴ Fischer, *Ber.*, **30**, 2220 (1897).

¹⁴⁵ Fischer, *Ber.*, **31**, 2550 (1898).

¹⁴⁶ Fischer, *Ber.*, **30**, 2226 (1897).

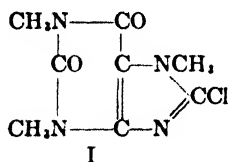
¹⁴⁷ Fischer, *Ann.*, **216**, 253 (1882).

¹⁴⁸ Fischer and Ach, *Ber.*, **28**, 3135 (1895).

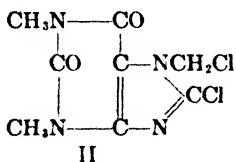
¹⁴⁹ Fischer and Ach, *Ber.*, **31**, 1980 (1898); Fischer and Clemm, *Ber.*, **31**, 2622 (1898).

¹⁵³ Fischer, *Ber.*, **30**, 2400 (1897); **28**, 2489 (1895).

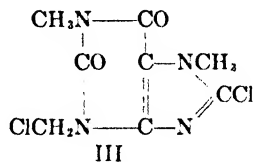
Caffeine¹⁵⁴ likewise can be demethylated to form paraxanthine, theophylline, and finally xanthine itself. For this purpose Fischer employed phosphorus pentachloride or a solution of chlorine in phosphorus oxychloride. By this means purine compounds represented by formulas I, II, III, and IV (below) were obtained. Hydrolysis of the chloromethyl groups in these purines, followed by reduction, led to the formation of the demethylated purines of the xanthine type.



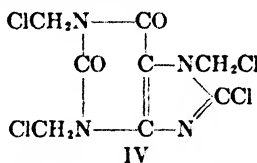
8-Chlorocaffeine



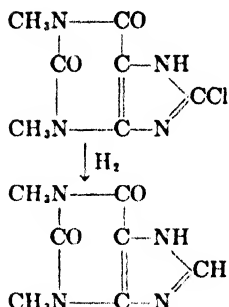
7',8-Dichlorocaffeine



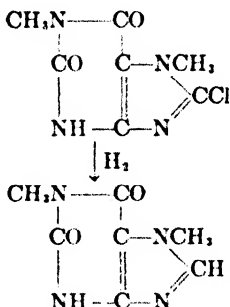
3',8-Dichlorocaffeine



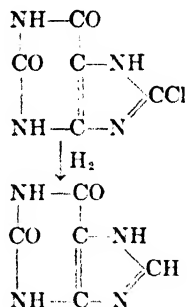
1',3',7',8-Tetrachlorocaffeine



Theophylline



Paraxanthine



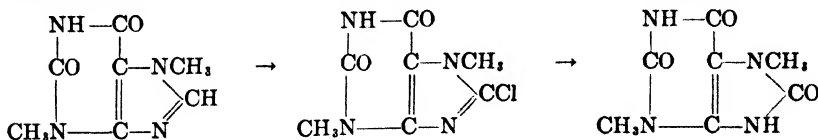
Xanthine

It is evident, therefore, that through the use of the halogenated purines obtained from uric acid and its derivatives Fischer was able to prepare xanthine and its derivatives quite readily. Having accomplished this task successfully, he next investigated the reverse change and was able to convert several of the xanthines into the corresponding uric acids. Through the action of halogens, the xanthines as a class undergo substitution in the 8-position.¹⁵⁵ Thus theobromine in chloroform solution when treated with chlorine yields 8-chlorotheobromine. The halogen

¹⁵⁴ Fischer and Ach, *Ber.*, **39**, 423 (1906).

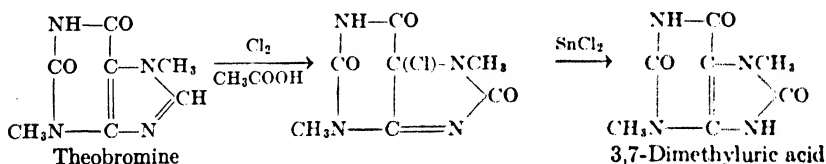
¹⁵⁵ Fischer, *Ann.*, **215**, 316 (1882); Fischer and Reese, *Ann.*, **221**, 336 (1883); Fischer and Ach, *Ber.*, **28**, 3141 (1895).

in the 8-position is quite resistant to the action of acids, but by means of alkali, replacement can be effected and 3,7-dimethyluric acid obtained in good yields.¹⁵⁶ In many cases, however, the action of alkali produces



profound changes in the purine molecule and as a result the method is of limited application.¹⁵⁷

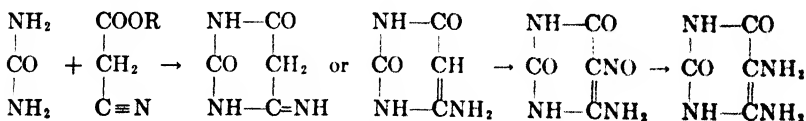
More recently Biltz¹⁵⁸ has introduced a new method of synthesis which although not general has proved very valuable in certain cases. By the action of chlorine in the presence of acetic acid, theobromine is converted directly into 3,7-dimethyl-5-chloroisouric acid. Upon reduction this compound is converted into 3,7-dimethyluric acid as follows:



In a somewhat similar manner theophylline¹⁵⁹ and caffeine¹⁶⁰ have been converted into the corresponding 1,3-dimethyluric acid and 1,3,7-trimethyluric acid, respectively.

Following closely Fischer's important work, Traube introduced his splendid method for the synthesis of purines.¹⁶¹ Of all available methods this is probably the most versatile and useful synthetic procedure.

The starting material of his method of synthesis is cyanoacetic acid or its ester which is condensed with urea to form cyanoacetylurea. When this is treated with alkali it is transformed into 4-aminouracil. By the action of nitrous acid a nitroso-aminouracil is formed which is readily reduced to 5,6-diaminouracil.



¹⁵⁶ Fischer, *Ber.*, **28**, 2480 (1895).

¹⁵⁷ Fischer, *Ber.*, **31**, 3272 (1898).

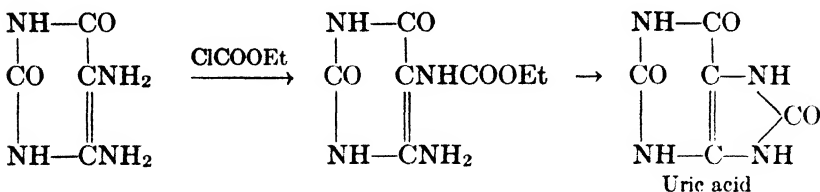
¹⁵⁸ Biltz and Damm, *Ann.*, **406**, 22 (1914).

¹⁵⁹ Biltz and Strufe, *Ann.*, **413**, 157 (1917).

¹⁶⁰ Biltz, *Ber.*, **43**, 3553 (1910).

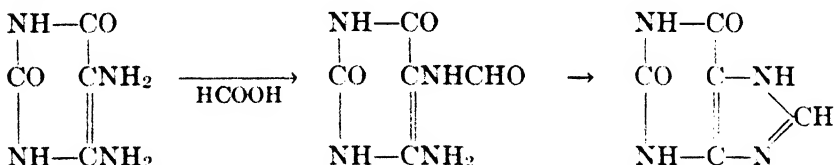
¹⁶¹ Traube, *Ber.*, **33**, 3045 (1900).

By the action of chlorocarbonic ester on this diamino derivative, a urethane results, the sodium salt of which is readily transformed into uric acid when heated.



Through the use of methylated pyrimidines the corresponding alkylated uric acids may be obtained easily.

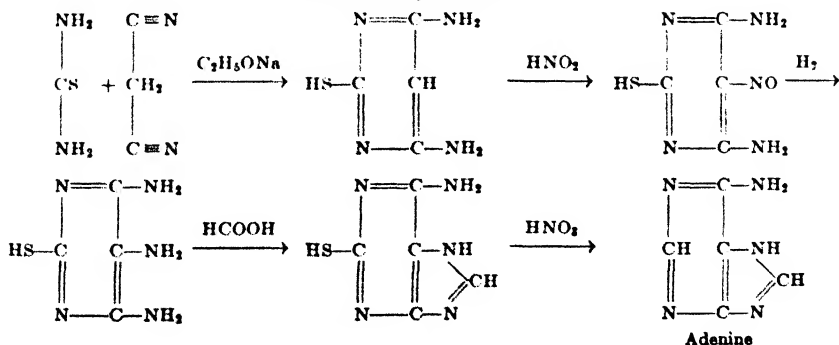
If formic acid is heated with the diaminopyrimidine, xanthine results.

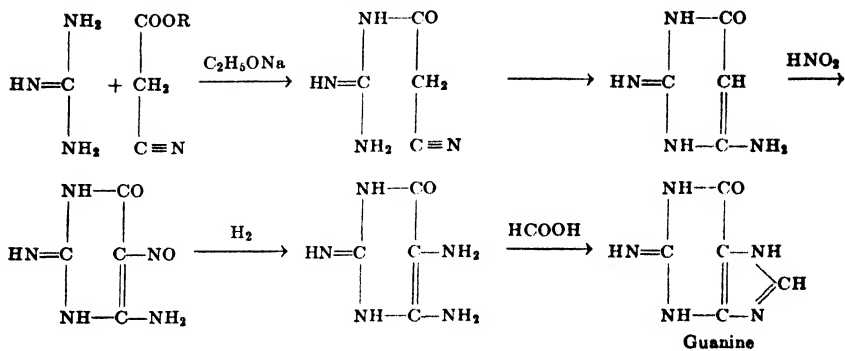
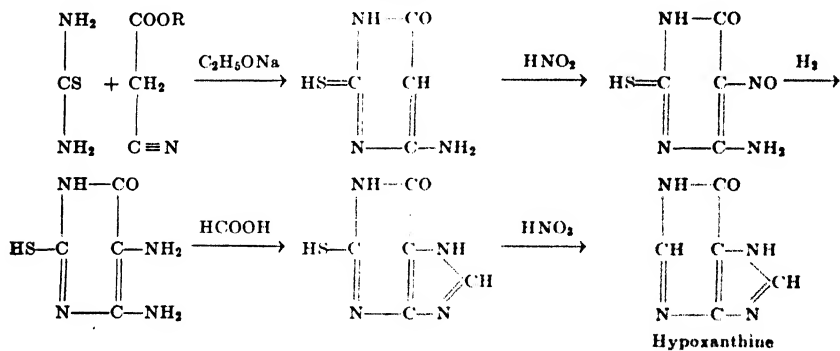
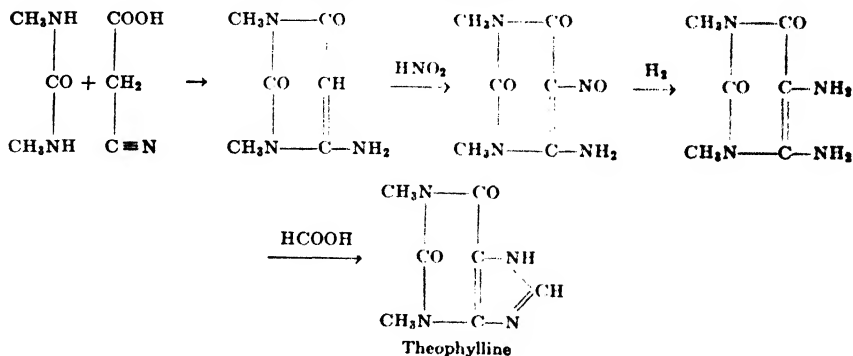


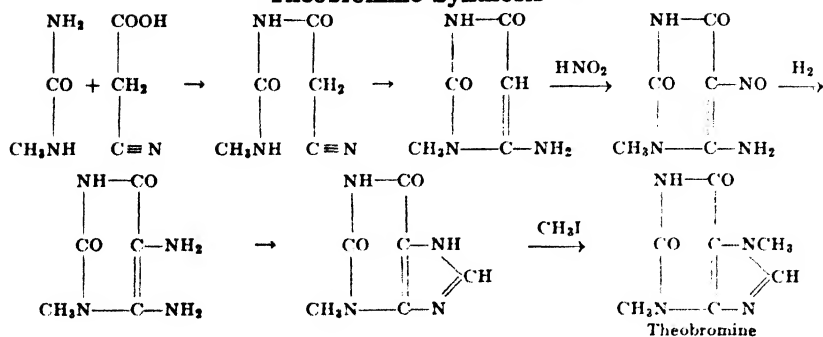
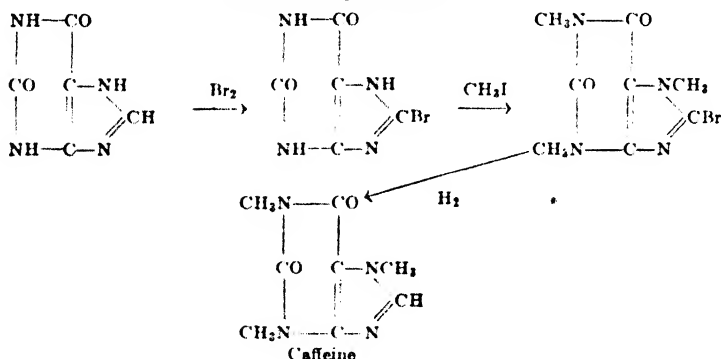
The utilization of alkylated pyrimidines permits the synthesis of alkylated xanthines.

The method is, therefore, directly applicable for the synthesis of various uric acids and xanthine derivatives. The replacement of urea by thiourea or guanidine is also possible. The following transformations demonstrate the utility of this synthetic procedure.

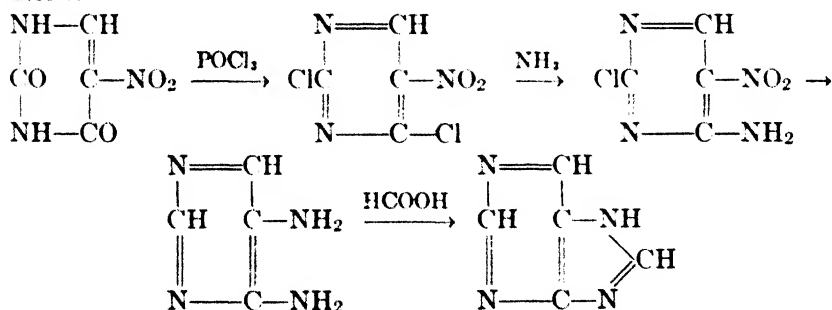
Adenine Synthesis¹⁶²



Guanine Synthesis¹⁶³Hypoxanthine Synthesis¹⁶⁴Theophylline Synthesis¹⁶⁵¹⁶³ Traube, *Ber.*, **33**, 1371 (1900).¹⁶⁴ Traube, *Ann.*, **331**, 64 (1904).¹⁶⁵ Traube, *Ber.*, **33**, 3052 (1900).

Theobromine Synthesis¹⁶⁶**Caffeine Synthesis**¹⁶⁷

In 1906 Isay¹⁶⁸ carried out the first complete synthesis of purine itself by starting with 5-nitrouracil. The method employed is outlined below.



Another method of preparing 2,8-dioxypurines was described by Johns¹⁶⁹ in 1909. 6-Amino-2-oxypyrimidines are nitrated and the re-

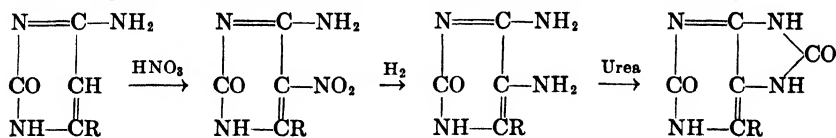
¹⁶⁶ Traube, *Ber.*, **33**, 3047 (1900).

¹⁶⁷ Traube, *Ber.*, **33**, 1371, 3035 (1900).

¹⁶⁸ Isay, *Ber.*, **39**, 250 (1906).

¹⁶⁹ Johns, *Am. Chem. J.*, **41**, 58 (1909); **45**, 79 (1911).

sulting nitropyrimidines are then reduced to 2-oxy-5,6-diaminopyrimidines. Upon heating these with urea, purines are obtained easily.



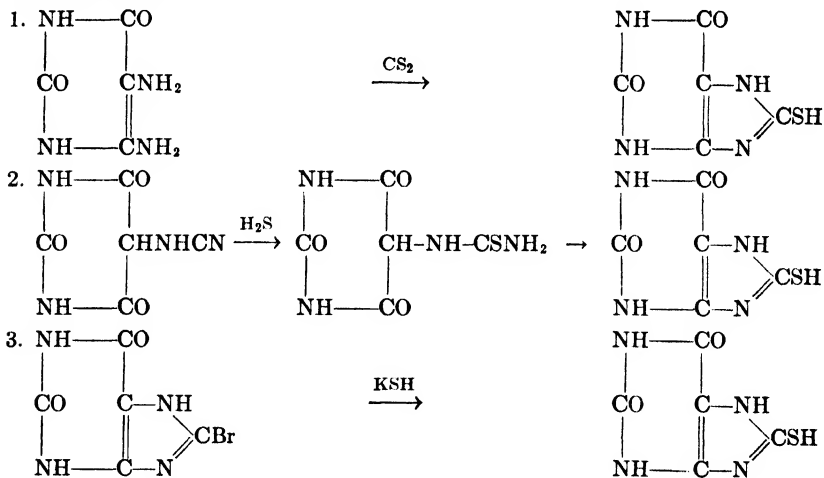
Among other reactions of the halogenated purines Fischer investigated their behavior with metallic hydrosulfides. By this means he was able to obtain thiopurines.¹⁷⁰ The most interesting of these sulfur purines are the 8-thio derivatives, since they may be readily converted to the corresponding xanthines by oxidation.¹⁷¹ Accordingly a number of procedures have been developed for their preparation, namely:

1. The heating of potassium urate or 5,6-diamino-2,4-dioxypyrimidine with carbon disulfide.¹⁷²

2. The conversion of cyanouramil into pseudothiouric acid with ammonium sulfide and finally to thiouric acid.¹⁷³

3. The interaction of 8-halogenated purines with potassium hydrosulfide.¹⁷⁴

4. The reaction of uramil with isothiocyanates¹⁷⁵ to form 9-substituted-8-thiolisoxanthines.



¹⁷⁰ Fischer, *Ber.*, **31**, 431 (1898).

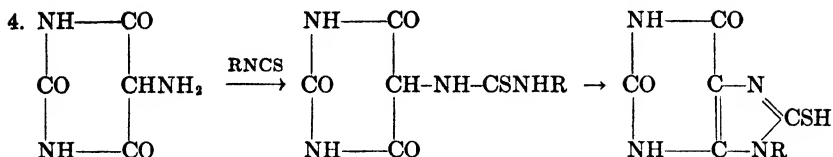
¹⁷¹ Fischer and Tüllner, *Ber.*, **35**, 2563 (1902); Fischer, *Ber.*, **32**, 486, (1899); Boehringer & Son, Ger. pat., 143, 725; [*Chem. Zentr.*, (II), 464 (1903)].

¹⁷² Boehringer & Sons, Ger. pat., 128,117; [*Chem. Zentr.*, (I), 548 (1902)]; Ger. pat., 142,468; [*Chem. Zentr.*, (II), 80 (1903)].

¹⁷³ Fischer and Tüllner, *Ber.*, **35**, 2563 (1902).

¹⁷⁴ Fischer, *Ber.*, **31**, 445 (1898).

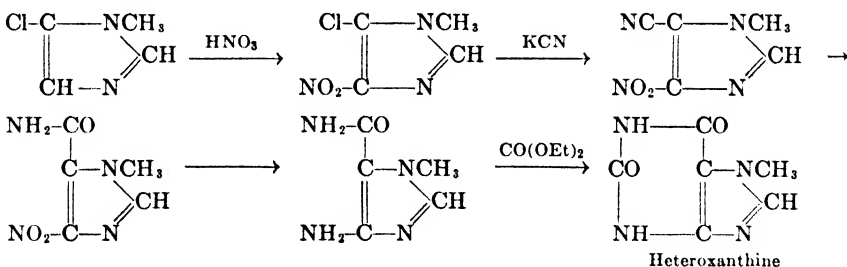
¹⁷⁵ Biltz and Strufe, *Ann.*, **423**, 200 (1921).



These thiopurines may be converted into the corresponding xanthines by treatment with nitrous acid,¹⁷¹ or as Biltz has demonstrated they may be reacted with sodium bicarbonate and iodine¹⁷⁶ to form 8-iodoxanthines, which upon reduction with hydriodic acid yield the corresponding xanthine or isoxanthine derivatives.

Although xanthine is one of the earliest known purines, it is probably the most difficult one to prepare in quantity. As early as 1864 Strecker¹⁷⁷ reported the reduction of uric acid with sodium amalgam to form xanthine, but later investigators were unable to corroborate his findings.¹⁷⁸ The later syntheses of this base by Fischer and Traube, though confirming the constitution, can hardly be described as practical methods of preparation. In 1897 Sundwik¹⁷⁹ reported the conversion of uric acid into xanthine by heating with formic acid. In 1928 Biltz¹⁸⁰ made a careful study of this reaction and developed a technique for obtaining xanthine from uric acid in satisfactory yields.

The first reported synthesis of purines from imidazoles was published by Sarasin and Wegmann¹⁸¹ in 1924. They used as their starting material dimethyloxamide which reacts with phosphorus pentachloride to yield 1-methyl-5-chloroimidazole. Their synthesis of heteroxanthine is expressed below.



The direct alkylation of uric acid has been shown to yield a variety of different alkyl derivatives depending upon the reagent employed and

¹⁷⁶ Biltz, *Ann.*, **426**, 237 (1922); Biltz and Beck, *J. prakt. Chem.*, [2] **118**, 149 (1928).

¹⁷⁷ Strecker, *Ann.*, **131**, 119 (1864).

¹⁷⁸ Fischer, *Ber.*, **17**, 329 (1884).

¹⁷⁹ Sundwik, *Z. physiol. Chem.*, **23**, 476 (1897); **26**, 131 (1899); **76**, 486 (1911)

¹⁸⁰ Biltz and Beck, *J. prakt. Chem.*, [2] **118**, 166 (1928).

¹⁸¹ Sarasin and Wegmann, *Helv. Chim. Acta*, **7**, 713 (1924); Wallach, *Ann.*, **214**, 257 (1882).

physical conditions under which the alkylation is conducted. Fischer¹⁸² and Biltz¹⁸³ have both studied very carefully the alkylation of the uric acids, and the reader is referred to their investigations for detailed information in this field. However, a brief survey of their findings is included to give at least a general idea of the reaction.

Biltz and Hermann demonstrated that the decreasing acidity of the hydrogen atoms in uric acid was in the order of positions 3, 9, 1, 7. They further showed that hydrogen atoms occupying positions 1 and 7 were so weakly acidic that uric acid behaved as a dibasic acid. It is apparent, therefore, that when metallic salts are produced the metal first replaces the hydrogen in position 3 and then the one in position 9. Subsequent treatment with alkyl halides simply replaces the metal by alkyl, hence uric acids alkylated in positions 3 and 9 result. Alkylation of the lead salts of the following uric acids with methyl iodide illustrates this principle.

LEAD SALT	ACID PRODUCED
Uric acid	3-Methyluric acid
7-Methyluric acid	3,7-Dimethyluric acid
7,9-Dimethyluric acid	3,7,9-Trimethyluric acid
3-Methyluric acid	3,9-Dimethyluric acid
1,3-Dimethyluric acid	1,3,9-Trimethyluric acid
3,7-Dimethyluric acid	3,7,9-Trimethyluric acid
3,9-Dimethyluric acid	3,9-Dimethyluric acid

When the dry potassium salts were allowed to interact with dimethyl sulfate similar results were obtained. On the other hand when dimethyl sulfate was allowed to react upon the acids dissolved in aqueous alkali the results were entirely different. In this case the less acidic hydrogen atoms in positions 1 and 7 were replaced. These changes are represented as shown below:

ALKALI SALT	ACID PRODUCED
3,7-Dimethyluric acid	1,3,7-Trimethyluric acid
7,9-Dimethyluric acid	1,7,9-Trimethyluric acid
3,9-Dimethyluric acid	1,3,7,9-Tetramethyluric acid.

Methyl iodide in the presence of alkali behaved similarly, although several exceptions were noted.

Diazomethane was found to be without action upon all uric acids substituted in both positions 3 and 9. It failed to react with 3,9-dimethyluric acid, 1,3,9-trimethyluric acid, and 3,7,9-trimethyluric acid. Uric acids not alkylated in position 9 are converted into 1,3,7-

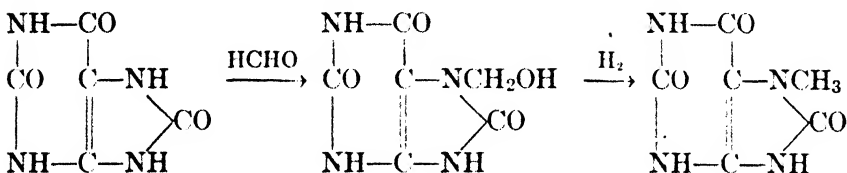
¹⁸² Fischer, *Ber.*, **32**, 461 (1899).

¹⁸³ Biltz and Max, *Ber.*, **53**, 2327 (1920); Biltz and Herrmann, *Ber.*, **54**, 1676 (1921).

trimethyl-8-methoxyxanthine upon treatment with this reagent. Thus uric acid and the 1,3-, 1,7-, 3,7-, and 1,3,7-methylated acids yielded 1,3,7-trimethyl-8-methoxyxanthine in every case.

Biltz also demonstrated that all purine derivatives alkylated in position 9 but with position 3 unoccupied were transformed into 1,7,9-trimethyl-6,8-dioxy-2-methoxypurine. The 9-, 1,9-, 7,9-, and 1,7,9-methylated acids behaved in this manner.

Another method of alkylation that has been employed is the action of formaldehyde upon uric acid to form the 7-hydroxymethyl derivative. This can be subsequently reduced to a 7-methyluric acid.¹⁸⁴



Inasmuch as various alkyluric acids may be directly synthesized by the Fischer method and the resulting alkyluric acids as well as uric acid itself may be further alkylated in accordance with the above principles, we are now able to prepare all the methyluric acids theoretically possible.

The direct action of alkylating agents upon the xanthines has attracted the attention of numerous investigators. We are indebted to Biltz,¹⁸⁵ however, for making a critical survey of the work in this field. As a result of his investigations, it at once became apparent that the order of decreasing acidity of the hydrogen atoms in xanthine replaceable by alkyl groups is 3,7,1. Thus 1-methylxanthine will alkylate to form 1,3-dimethylxanthine and finally 1,3,7-trimethylxanthine. In similar manner 3-methylxanthine first yields 3,7-dimethylxanthine and ultimately 1,3,7-trimethylxanthine. The direct alkylation of xanthine or its salts with alkyl halides is very difficult and impractical. The alkylxanthines or their halogen derivatives, however, alkylate further with ease when alkyl halides are employed.

Dimethyl sulfate very readily and completely converts xanthine or its salts into caffeine. The action of diazomethane was found to be exceedingly slow. Complete alkylation to caffeine finally resulted, however, when this reagent was employed.

¹⁸⁴ Fischer and Ach, *Ber.*, **32**, 250 (1899); Biltz and Herrmann, *Ber.*, **54**, 1693 (1921).

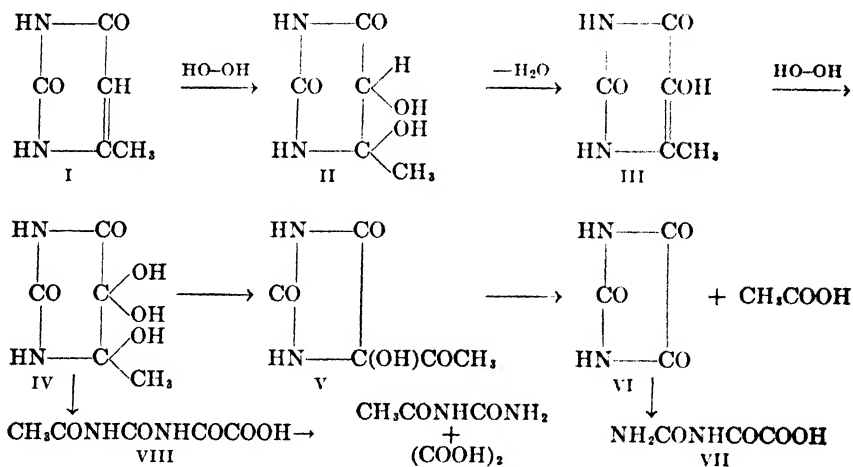
¹⁸⁵ Biltz and Beck, *J. prakt. Chem.* [2] **118**, 198 (1928).

OXIDATION OF PYRIMIDINES AND PURINES

The course of the oxidation of both pyrimidines and purines depends to a large extent on the pH of the medium in which the reaction is carried out. Since this factor exerts such a great influence on the type of product obtained, the oxidation processes have been classified as those occurring in (a) alkaline solution and (b) acid solution.

Alkaline Oxidation of Pyrimidines

Hydrolytic Oxidation. The majority of the oxidizing agents used in alkaline solution bring about not only oxidation, but also cleavage of the pyrimidine ring with the formation of urea or substituted urcas. Alkaline permanganates, for example, oxidize thymine to urea,¹⁸⁶ and cytosine to biuret,¹⁸⁷ reactions which were used by Kossel and Steudel to prove the structure of thymine and cytosine. By oxidation of 6-methyluracil* (I) with alkaline permanganate, Behrend obtained acetylurea and oxalic acid if the reaction took place in the cold, but oxaluric acid (VII) and acetic acid if the oxidizing mixture was heated.^{188, 189} Behrend postulated¹⁸⁹ that the primary product of the oxidation of 6-methyluracil was the glycol (II) formed by the addition of two hydroxyl groups to the double bond in positions 5 and 6. Behrend's conception of the mechanism of the entire oxidation process is represented below.



¹⁸⁶ Steudel, *Z. physiol. Chem.*, **30**, 539 (1900); **32**, 241 (1901).

¹⁸⁷ Kossel and Steudel, *ibid.* **38**, 53 (1903).

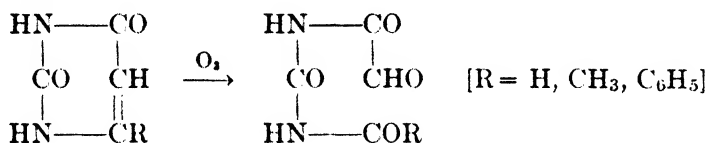
* 4-Methyluracil, according to old nomenclature.

¹⁸⁸ Behrend and Dietrich, *Ann.*, **309**, 262 (1899).

¹⁸⁹ Behrend and Grünewald, *Ann.*, **223**, 180 (1902).

The evidence for this formulation of the process is based on the following facts. The oxymethyluracil (III) was isolated¹⁸⁹ and found to give the same oxidation products as the original 4-methyluracil. The trihydroxy derivative (IV), while not isolated from the reaction, was synthesized by Behrend and Osten¹⁹⁰ and shown to give on oxidation the same products as 6-methyluracil. The concept of parabanic acid (VI) as an intermediate product in the formation of oxaluric acid (VII) explains the fact that the 3,6-dimethyl- and the 1,6-dimethyluracils gave the same 1-methyloxaluric acid on oxidation. Moreover, the oxidation of 1,3,6-trimethyluracil yielded products in accordance with the scheme.¹⁹¹

A series of urea derivatives similar to those resulting from oxidation with permanganates were obtained by Johnson and Flint¹⁹² through the action of ozone on uracil and related compounds. For example, formylglyoxylurea was obtained from uracil on ozonization, and the corresponding acetyl and benzoyl derivatives from 6-methyl- and 6-phenyluracils, respectively.



Although these ozonizations were conducted in acetic acid solution, the reactions have been discussed at this point because the products obtained resemble those resulting from alkaline rather than from acid oxidations. It will be seen that acetic acid functions in the same way in the oxidation of purines, exerting an influence similar to that of alkalis, but entirely different from that of strong mineral acids.

Another oxidizing agent which brings about hydrolysis of the pyrimidine ring is iodine in alkaline solution. Urea has been obtained from uracil, 6-methyluracil, cytosine, and thymine by heating their aqueous alkaline solutions with iodine.¹⁹³

An alkaline oxidizing agent which is of biological interest because of the mildness of the conditions employed consists of the system ferrous salts, sodium bicarbonate, and oxygen.¹⁹⁴ Pfaltz and Baudisch found that when uracil or 6-methyluracil was treated with this mixture

¹⁸⁹ Behrend and Osten, *Ann.*, **343**, 133 (1905).

¹⁹¹ Behrend and Fricke, *Ann.*, **337**, 255 (1903).

¹⁹² Johnson and Flint, *J. Am. Chem. Soc.*, **53**, 1077, 1082 (1931).

¹⁹³ Bass and Baudisch, *ibid.*, **46**, 181 (1924).

¹⁹⁴ Pfaltz and Baudisch, *ibid.*, **45**, 2972 (1923).

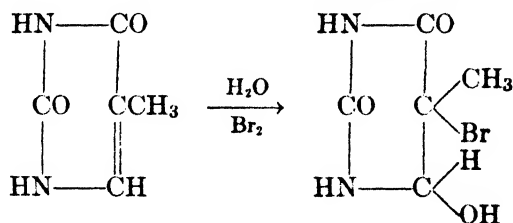
oxidation occurred in the cold, followed by hydrolysis with the splitting off of urea upon warming. When thymine was subjected to the same catalytic oxidation, acetol and pyruvic acid were isolated in addition to urea.

Coupling Oxidation. Oxidation reactions which involve the union of pyrimidine molecules rather than the hydrolysis of the ring have been brought about by potassium ferricyanide in alkaline solution. When isobarbituric acid, for example, is treated with this reagent, a colored dicyclic compound, 6,6'-diisobarbituric acid, is obtained.¹⁹⁵ Oxidation of 5-aminouracil in a similar way yields another unique colored product, the tricyclic diuracil-pyridazine.¹⁹⁶

Acid Oxidation of Pyrimidines

Though the pyrimidine molecule has been shown to be extremely susceptible to decomposition in alkaline media, it is remarkably stable in acid solutions. Uracil, for example, is unchanged by the action of cold fuming sulfuric acid, the reagent used in the Davidson and Baudisch method¹⁹⁷ of preparing this pyrimidine. Accordingly, when pyrimidines are affected by acidic oxidants, there is usually no resultant hydrolysis of the ring, but merely an addition of oxygen or oxygen-containing radicals to the double bond in positions 5 and 6.

Bromine water is an acidic oxidant of this type which brings about the addition of hypobromous acid to 2,4-dioxypyrimidines. Thymine, for example, yields 5-bromo-6-oxyhydrothymine on contact with bromine.¹⁹⁸ With uracil and derivatives having no substituent in position



5, the addition of hypobromous acid is accompanied by the substitution of a bromine atom in position 5, giving dibromoöxyhydrouracils.^{199,200}

¹⁹⁵ Davidson and Baudisch, *J. Biol. Chem.*, **64**, 620 (1925).

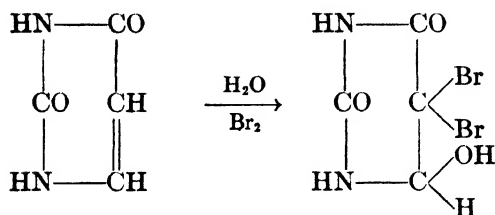
¹⁹⁶ Baudisch and Davidson, *ibid.*, **71**, 498 (1927).

¹⁹⁷ Davidson and Baudisch, *J. Am. Chem. Soc.*, **48**, 2379 (1926).

¹⁹⁸ Jones, *Z. physiol. Chem.*, **29**, 20 (1900).

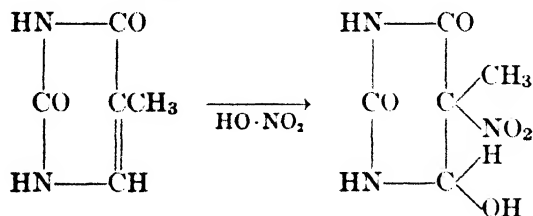
¹⁹⁹ Behrend, *Ann.*, **229**, 18 (1885).

²⁰⁰ Wheeler and Johnson, *J. Biol. Chem.*, **3**, 187 (1907).



These reactions with bromine water are the basis of the Wheeler and Johnson color test²⁰⁰ for uracil and cytosine, and the Johnson and Harkins test²⁰¹ for thymine and 5-methyleytosine. The former consists in the transformation of uracil or cytosine into dibromoöxyhydrouracil and the conversion of this product into the purple barium salt of dialuric acid by hydrolysis with barium hydroxide. The bromoöxyhydrothymine, obtained from either thymine or 5-methyleytosine and bromine water, does not give dialuric acid by hydrolysis with barium hydroxide, but is decomposed into urea, acetol, and pyruvic acid which may be separated and identified by the method of Johnson and Baudisch.²⁰² This procedure, therefore, furnishes a convenient way of distinguishing thymine and 5-methyleytosine from uracil and cytosine.

The oxidizing action of nitric acid on pyrimidines varies with the type of pyrimidine used. Addition of oxy and nitro groups to the double bond in positions 5 and 6 is the reaction which takes place with 2,4-dioxypyrimidines having substituents in position 5.^{203, 204} Thymine, for example, when treated with concentrated nitric acid, yields 6-oxy-5-nitrohydrothymine, which exists in two stereoisomeric forms. Nitric acid reacts here in the form $\text{HO} \cdot \text{NO}_2$ or in a manner similar to $\text{HO} \cdot \text{Cl}$ which adds at the double bond in the pyrimidine cycle.



Uracil, on the other hand, and derivatives of uracil having no substituent in position 5 react with fuming nitric acid to form 5-nitro derivatives.²⁰³

²⁰¹ Harkins and Johnson, *J. Am. Chem. Soc.*, **51**, 1237 (1929).

²⁰² Johnson and Baudisch, *ibid.*, **43**, 2672, 2673 (1921).

²⁰³ Johnson, *Am. Chem. J.*, **40**, 21 (1908).

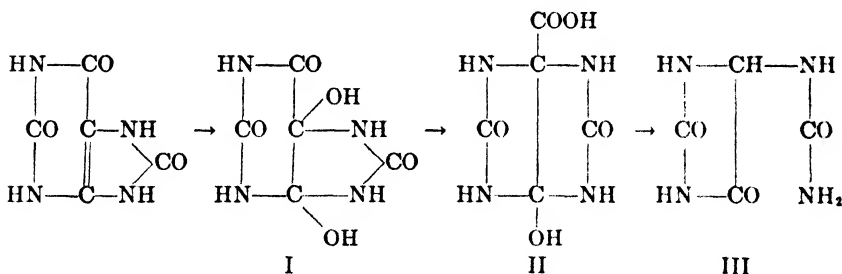
²⁰⁴ Johnson and Clapp, *J. Biol. Chem.*, **5**, 53 (1908).

This difference in the reaction of uracil and thymine toward nitric acid was utilized by Johnson as the basis of a convenient method of separating these two pyrimidines.²⁰⁵

Alkaline Oxidation of Purines

The most characteristic product of the alkaline oxidation of uric acid is allantoin, which was first obtained by Wöhler and Liebig²⁰⁶ through the use of lead peroxide in boiling water. Many other oxidizing agents, such as potassium permanganate,^{207,208,209} hydrogen peroxide,^{210,211} air in the presence of catalysts,^{212,213} potassium persulfate,²⁰⁹ and nitrous acid,²⁰⁸ also give allantoin in alkaline media. Biltz has shown²⁰⁹ that the designation "alkaline" should include not only strictly basic, but also neutral or weakly acid, media, all of which give markedly different products from strongly acid solutions.

A mechanism to explain the oxidation of uric acid to allantoin was first suggested by Behrend.¹⁸⁹ Behrend assumed that the initial stage in the oxidation of uric acid, like that of 6-methyluracil, consisted in the formation of the glycol (I), which was then transformed by hydrolysis and ring closure into glycoluriloxycarbonic acid (II). Subsequent hydrolysis of the latter substance would give allantoin (III).



This mechanism accounts for the products obtained by Fischer and Ach²¹⁴ upon oxidation of certain methylated uric acids. Both 1-methyl- and 7-methyluric acids were found to give 3-methylallantoin, while

²⁰⁵ Johnson, *ibid.*, **4**, 407 (1908).

²⁰⁶ Wöhler and Liebig, *Ann.*, **26**, 285 (1838).

²⁰⁷ Claus, *Ber.*, **7**, 227 (1874).

²⁰⁸ Behrend, *Ann.*, **333**, 146 (1904).

²⁰⁹ Biltz and Schauder, *J. prakt. Chem.*, [2] **106**, 114, 115, 122, 123 (1923).

²¹⁰ Venable, *J. Am. Chem. Soc.*, **40**, 1099 (1918).

²¹¹ Wieland and Macrae, *Z. physiol. Chem.*, **303**, 83 (1931).

²¹² Frèrejacque, *Compt. rend.*, **193**, 860 (1931).

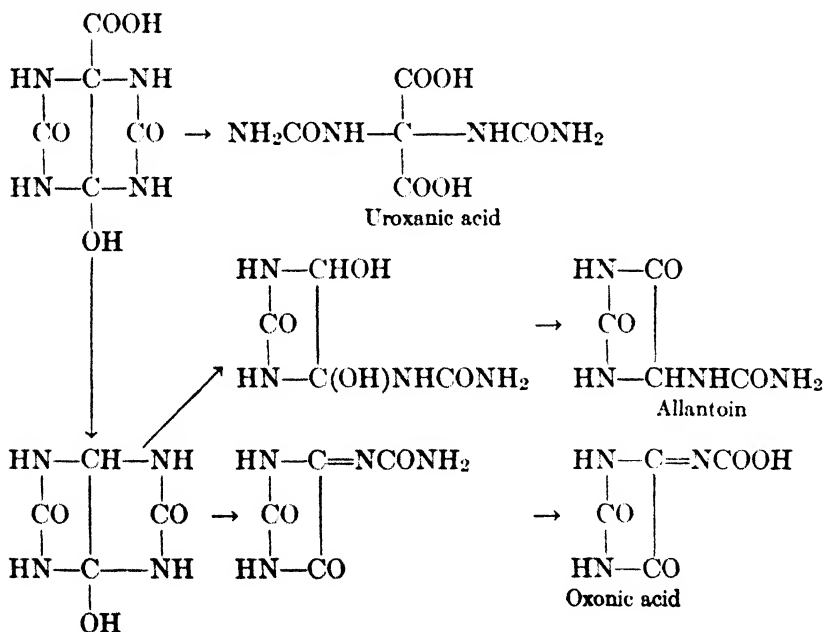
²¹³ Piaux, *ibid.*, **178**, 782 (1924).

²¹⁴ Fischer and Ach, *Ber.*, **32**, 2723 (1899).

both 3-methyl- and 9-methyluric acids yielded 1-methylallantoin. These results are explained by the symmetry of the glycolurilcarbonic acid (II), which would give only two monomethyl derivatives and consequently only two monomethylallantoins on hydrolysis.

Allantoin is not, however, the sole product of the alkaline oxidation of uric acid. Three other substances frequently obtained are uroxic acid, ²¹⁵ oxonic acid, ²¹⁶ and oxaluric acid. ²⁰⁹ A summary of the conditions which lead to the formation of each of these products has been given by Biltz and Schiemann. ²¹⁷ When uric acid is oxidized in alkaline solution the resulting liquor gives (a) allantoin, if acidified with acetic acid and evaporated, (b) the potassium salt of uroxic acid if evaporated with strong alkali, (c) the acid potassium salt of oxonic acid if acidified with acetic acid and allowed to stand, and (d) the potassium salt of oxaluric acid if oxidized further in acetic acid solution.

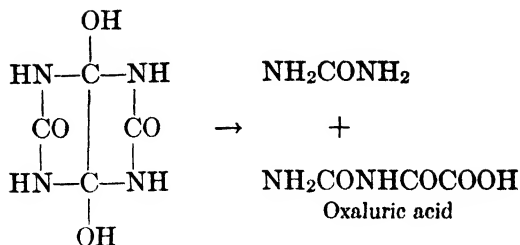
Behrend showed that the formation of uroxic acid as well as of allantoin could be explained by the assumption that the glycoluriloxycarbonic acid was capable of hydrolysis in two ways. Behrend's postulations, extended by Biltz ²¹⁷ to include also an explanation of the production of oxonic and oxaluric acids, are shown below.



²¹⁵ Biltz and Robl, *Ber.*, **53**, 1950 (1920).

²¹⁶ Biltz and Robl, *Ber.*, **53**, 1967 (1920).

²¹⁷ Biltz and Schiemann, *J. prakt. Chem.*, [2] **113**, 77 (1926).



Two additional end products, carbonylbiurea and cyanuric acid, are obtained²¹⁸ by alkaline oxidation of uric acid, particularly when hydrogen peroxide is the oxidizing agent. The former is believed to be formed from the original glycoluriloxycarbonic acid; the latter may be a secondary product formed by further decomposition of the carbonylbiurea of allantoin.²¹⁰ It is of interest to note that oxidation experiments with hydrogen peroxide carried out under physiological conditions (at a pH of 8.0 and a temperature of 37°) yielded a similar series of products as experiments at a higher alkalinity and temperature: namely, allantoin, carbonylbiurea, cyanuric acid, urea, oxalic acid, and ammonia.²¹¹

The conception of the glycoluriloxycarbonic acid as the precursor of these various oxidation products has been recently substantiated by the isolation of a silver salt of this compound by oxidation of uric acid with cold alkaline permanganate.²¹⁹ This salt, on hydrolysis, yields allantoin and uroxanic acid. The original postulations of Behrend and of Biltz which represented uric acid glycol as the initial product formed by the attack of alkaline oxidizing agents on uric acid have been modified by evidence gained from studies of the glycol. This substance, synthesized by Biltz and Heyn²²⁰ from alloxan and urea, yields on alkaline hydrolysis no allantoin or any of the other products characteristic of the oxidation of uric acid.^{221, 222} Hence Biltz has concluded²²³ that the glycoluriloxycarbonic acid, rather than the uric acid glycol, is the primary intermediate product.

Acid Oxidation of Purines

The chief products of the oxidation of uric acid in an acid medium are alloxan and urea. Alloxan was first obtained by Brugnatelli²²⁴

²¹⁸ Schittenhelm and Warnat, *Z. physiol. Chem.*, **171**, 174 (1927).

²¹⁹ Schuler and Reindel, *ibid.*, **208**, 251 (1932).

²²⁰ Biltz and Heyn, *Ber.*, **45**, 1677 (1912); **47**, 459 (1914).

²²¹ Behrend and Zieger, *Ann.*, **410**, 338 (1915).

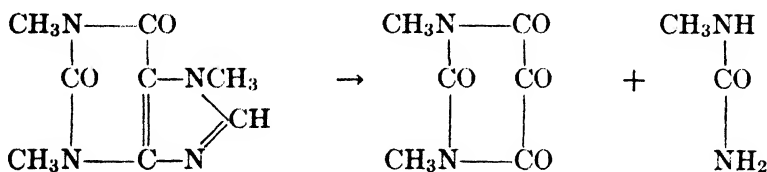
²²² Biltz and Heyn, *Ann.*, **413**, 56 (1916).

²²³ Biltz and Max, *Ber.*, **54**, 2457 (1921).

²²⁴ Brugnatelli, *Phil. Mag.*, **52**, 30 (1818); *Ann. chim. phys.*, **8**, 201 (1817).

through the treatment of uric acid with nitric acid or chlorine water. Evidence as to the structure of alloxan and its relation to other ureides was furnished by the monumental researches of Wöhler and Liebig on the oxidation of uric acid.²⁰⁶

This transformation of uric acid to alloxan and urea was later used by Fischer²²⁵ in the determination of the structure of various naturally occurring purines. For example, the oxidation of caffeine to dimethylalloxan and monomethylurea by chlorine water gave information as to the position of the methyl groups in caffeine.



The mechanism of the acid oxidation of uric acid to alloxan, like that of the alkaline oxidation to allantoin, was originally thought to involve the initial formation of uric acid glycol. This hypothesis was based on Biltz's discovery that a substance isolated by Fischer²²⁶ from the oxidation of 7,9-dimethyluric acid was identical with the glycol of 7,9-dimethyluric acid obtained by synthesis.²²⁷ Similarly, the glycol of 1,3-dimethyluric acid obtained by oxidation of theophylline was identical with the synthetic product prepared from dimethylalloxan and urea.²²⁸

But further investigations of the purine glycols showed that they did not give alloxan or its derivatives on decomposition. Unsubstituted uric acid glycol, for example, could not be converted into alloxan.²²⁹ Moreover, 9-methyluric acid glycol on hydrolysis gave not monomethylurea, but unsubstituted urea,²⁰⁹ showing that the pyrimidine, and not the glyoxalone ring was split. Since uric acid derivatives are known to yield alloxan derivatives through acid oxidation, the assumption that the glycols are intermediate products in all such processes is open to question.

An alternative mechanism has been suggested by Biltz and Schauder²⁰⁹ on the basis of their experience with pseudouric acids. When uric acid is chlorinated in a solution of acetic acid containing one mole of water, 5-chloropseudouric acid is obtained, which in the presence of more water is changed at once to 5-oxypseudouric acid.

²²⁵ Fischer, *Ann.*, **215**, 257 (1882).

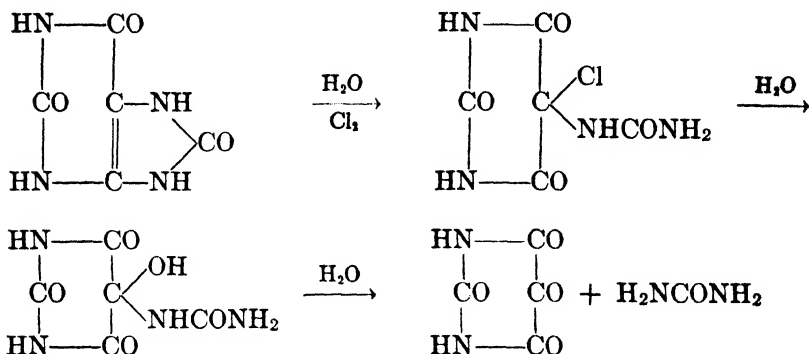
²²⁶ Fischer, *Ber.*, **17**, 1780 (1884).

²²⁷ Biltz, *Ber.*, **43**, 1513 (1910).

²²⁸ Biltz and Strufe, *Ann.*, **404**, 131 (1914).

²²⁹ Biltz and Heyn, *Ber.*, **45**, 1677 (1912).

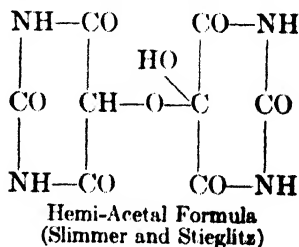
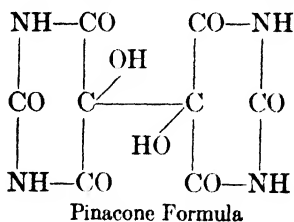
The latter substance can be rapidly hydrolyzed to alloxan and urea by warming its aqueous solution. The formation of alloxan by the oxidation of uric acid with chlorine water is, therefore, assumed to proceed through 5-chloropseudouric acid and 5-oxypseudouric acid.



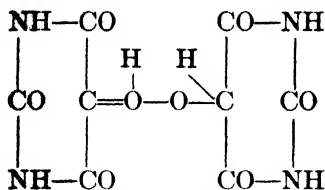
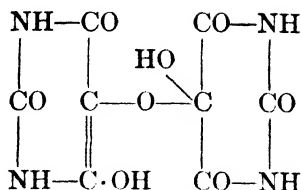
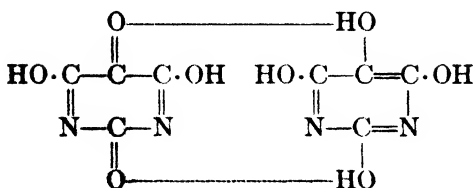
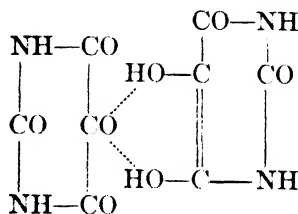
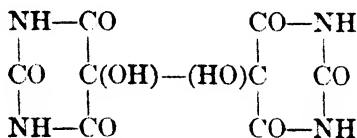
In addition to alloxan, parabanic acid is frequently obtained by energetic oxidation of uric acid in acid media. Since alloxan can be changed to parabanic acid by oxidation, the formation of the latter substance is thought to be due to a secondary reaction of the oxidant on the alloxan.²⁰⁹

Two other products resulting from the oxidation of uric acid are of historical and practical importance, namely, alloxantine and murexide. Alloxantine was obtained by Wöhler and Liebig²⁰⁶ when dilute, rather than concentrated, nitric acid was used to oxidize uric acid. The constitution of this pyrimidine is still unsettled. Various formulas which have been proposed to represent its structure follow.²³⁰

CONSTITUTIONAL FORMULAS PROPOSED FOR ALLOXANTINE



²³⁰ *The Alloxantine Question*: Behrend and Friederichs, *Ann.*, **344**, 1 (1906); Schlenk and Thal, *Ber.*, **46**, 2840 (1913); Retinger, *J. Am. Chem. Soc.*, **39**, 1059 (1917); Billman and Bentzon, *Ber.*, **51**, 522 (1918); Biltz and Paetzold, *Ann.*, **433**, 64 (1923); Hantsch, *Ber.*, **54**, 1271 (1921); Michaelis, *Chem. Rev.*, **16**, 243 (1935).

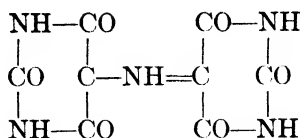
Oxonium Formula
(Richter)Hemi-Acetal Formula
(Piloty)Quinhydrone Formula
(Willstätter)Hydrate Formula
(Biltz)Trivalent Carbon
Formula
(Hantzsch)*Literature references.**Pinacone formula:*Hantzsch, *Ber.*, **54**, 1267 (1921); Retinger, *J. Am. Chem. Soc.*, **39**, 1059 (1917).*Hydrate formula:*Biltz, *Ann.*, **433**, 64 (1923).*Hemi-Acetal formula:*Slimmer and Stieglitz, *Am. Chem. J.*, **31**, 661 (1904); Piloty, *Ann.*, **333**, 62 (1904).*Quinhydrone formula:*Willstätter and Piccard, *Ber.*, **41**, 1464 (1908); Abderhalden, "Biochemische Handlexikon," Springer, Berlin (1911), Vol. IV, p. 1163.*Oxonium formula:*Richter, *Ber.*, **44**, 2155 (1911); Büllmann and Bentzon, *Ber.*, **51**, 522 (1918).

Murexide was discovered by Prout,²³¹ who observed that the solution obtained by oxidizing uric acid with nitric acid became deep violet when treated with ammonia. The colored substance, called murexide, is thought to be an ammonium salt of purpuric acid, and is used as a color test for uric acid and related purines.²³² The various formulas which

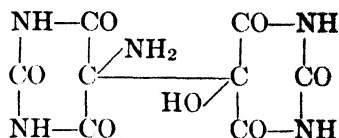
²³¹ Prout, *Phil. Trans.*, 420 (1818).²³² (a) Fischer, *Ber.*, **30**, 2236 (1897), (b) *The Murexide Question*: Wöhler and Liebig, *Ann.*, **26**, 319 (1838); Strecker, *Ann.*, **123**, 363 (1862); Beilstein, *Ann.*, **107**, 176 (1858); Matignon, *Ann. chim. phys.*, [6] **28**, 289 (1893); Hartley, *J. Chem. Soc.*, **87**, 1791 (1905);

have been proposed to represent the structure of murexide are shown below.

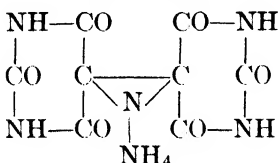
CONSTITUTIONAL FORMULAS PROPOSED FOR
PURPURIC ACID AND MUREXIDE



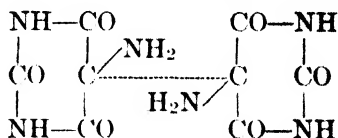
Purpuric Acid



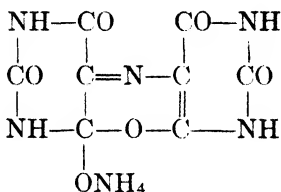
Purpuric Acid



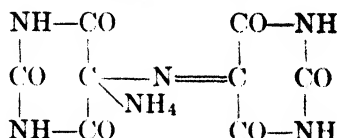
Matignon's Murexide
Formula



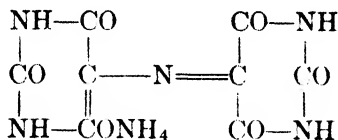
Murexide Free
Radical Structure



Piloty's Murexide
Formula (1)



Piloty's Murexide
Formula (2)



Slimmer and Stieglitz
Murexide Formula

Literature references.

Purpuric acid:

- Meyer and Jacobson, "Lehrbuch der organischen Chemie," deGruyter, Berlin and Leipzig (1920), Vol. II, part 3, p. 1217.
 Bernthsen, "Kurzen Lehrbuch der organischen Chemie," Vieweg und Sohn, Braunschweig (1924), p. 337.
 Liebig and Wöhler, *Ann.*, **26**, 319 (1838).

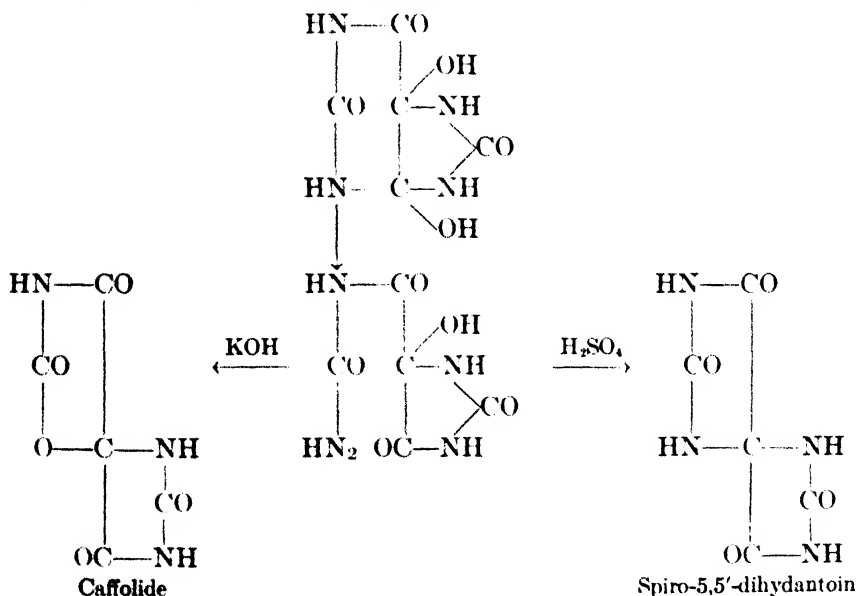
Piloty, *Ann.*, **333**, 22 (1904); Möhlau, *Ber.*, **37**, 2686 (1904); Slimmer and Stieglitz, *Am. Chem. J.*, **31**, 661 (1904); Traube, *Ber.*, **44**, 3145 (1911); Piloty and Finckh, *Ann.*, **333**, 41 (1904); Techow, *Ber.*, **27**, 3083 (1894); Biltz and Damm, *Ber.*, **45**, 3673 (1912); **46**, 3668 (1913); Hartman and Sheppard, "Organic Syntheses," John Wiley & Sons, New York City (1932), Vol. 12, p. 84; Kuhn and Lyman, *Ber.*, **69**, 1547 (1936); Davidson and Epstein, *J. Org. Chem.*, **1**, 305 (1936); Davidson, *J. Am. Chem. Soc.*, **58**, 1821 (1936).

Fritzsche, *Ann.*, **32**, 316 (1839).
 Beilstein, *Ann.*, **107**, 176 (1858).
 Gregory, *Ann.*, **33**, 334 (1840).
 Slimmer and Stieglitz, *Am. Chem. J.*, **31**, 661 (1904).

Murexide:

Möhlau and Litter, *J. prakt. Chem.*, [2], **73**, 449 (1906); *Ber.*, **37**, 2686 (1904).
 Matignon, *Ann. chim.*, [3] **28**, 347 (1893).
 Slimmer and Stieglitz, *Am. Chem. J.*, **31**, 661 (1904).
 Piloty, *Ann.*, **333**, 53 (1904).
 Abderhalden, "Biochemische Handlexikon," Springer, Berlin (1911), Vol. IV, p. 1166.

Reference has already been made to the fact that the purine glycols do not yield the same hydrolytic products as are obtained by oxidation and hydrolysis of the purines. Yet because of the close structural relationship between the purine glycols and the purines, a summary of the unusual decomposition products of the glycols is included in this survey. A series of investigations initiated by Fischer and extended by Biltz and his collaborators have demonstrated that the purine glycols are decomposed by alkalis or acids to give (a) caffolides, or (b) spirohydantoin, which gives in brief the decomposition of uric acid glycol.^{233,234}



In conclusion, attention is directed to the similarity in the behavior of the uracil molecule and the pyrimidine nucleus of the uric acid mole-

²³³ Biltz and Heyn, *Ann.*, **413**, 56 (1916).

²³⁴ Biltz, Heyn, and Bergius, *Ann.*, **413**, 79 (1916).

cule on oxidation. Uracil undergoes hydrolysis of the ring when treated with alkaline oxidizing agents, but is oxidized at the double bond in positions 5 and 6 without cleavage of the ring by acid oxidizing agents. Similarly, when uric acid is oxidized in alkaline solution, the pyrimidine ring is broken and the glyoxalone allantoin is produced, but when oxidized in acid solution, the glyoxalone ring is destroyed and the pyrimidine alloxan is obtained.

REDUCTION AND HYDROGENATION OF PYRIMIDINES AND PURINES*

The following selections from the chemical literature on the reduction and hydrogenation of pyrimidine and purine derivatives may be considered as quite thoroughly representative of the subject matter and methods of experimentation. The effects of reducing agents and hydrogenation conditions upon pyrimidine or purine derivatives have been divided into two types. The first includes all those reactions which affect or alter the linking of the atoms in the nucleus. The second includes those reactions which replace by hydrogen an atom directly attached to one of the atoms in the nucleus.

Nuclear Reduction

Illustrative examples follow of those reductions and hydrogenations which affect or alter the linking of the atoms in the nucleus, i.e., reduction of double bonds and cleavage of bonds between any two carbon or nitrogen and carbon atoms.

Reductions by Sodium Amalgam, Sodium and Ethyl Alcohol, Hydriodic Acid, Zinc and Acids, etc. E. von Meyer²³⁵ apparently first observed that the pyrimidine nucleus is cleaved either by the action of sodium amalgam in acid solution or by sodium and ethyl alcohol. By such reactions he found that 2,6-diethyl-4-amino-5-methylpyrimidine (V), was reduced to ammonia, propionaldehyde, and a basic oil. Biginelli²³⁶ reported that the 2-ketotetrahydropyrimidine (VI), when treated with sodium amalgam yields the corresponding hexahydropyrimidine derivative. Byk²³⁷ was next to study the cleavage of the pyrimidine nucleus by the action of sodium and ethyl alcohol, and he definitely demonstrated that 6-methylpyrimidine (VII), reduces to 1,3-diamino-

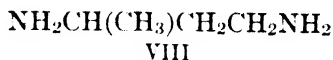
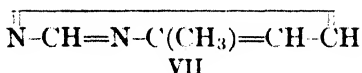
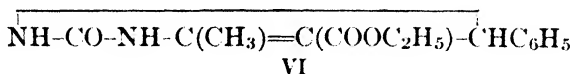
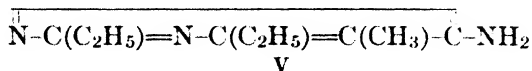
* As commonly differentiated from reduction, hydrogenation herein refers to those methods which involve reactions of organic compounds with molecular hydrogen, and in these cases in the presence of a catalyst.

²³⁵ v. Meyer, *J. prakt. Chem.*, [2] **39**, 262 (1889).

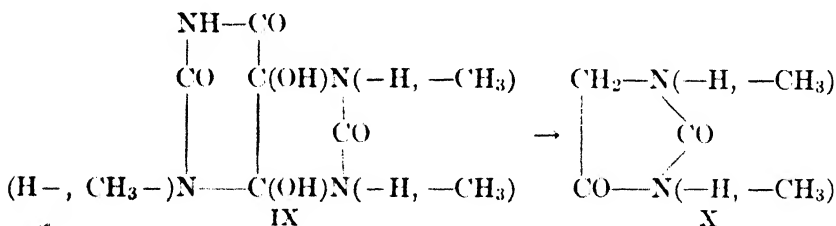
²³⁶ Biginelli, *Gazz. chim. ital.*, [1] **23**, 366 (1893).

²³⁷ Byk, *Ber.*, **36**, 1917 (1903).

butane (VIII) and that 2-methylmercaptopyrimidine²³⁸ is reduced by sodium and ethyl alcohol to 1,3-diaminopropane and methylthioformate presumably proceeding through the hexahydropyrimidine derivative. Johnson and Joyce²³⁹ applied this sodium and ethyl alcohol reduction technique to six pyrimidines of the 2-mercapto-4-ketopyrimidine type and also to 2-thio-4-ketopyrimidine. In each case an acyclic 1,3-diamino compound was formed.



Biltz²⁴⁰ found that uric acid glycols could not be reduced to uric acids, but, with one exception, they could be reduced to hydantoins by hydriodic acid, as shown in formulas IX and X. Later²⁴¹ he obtained 7,9-dimethyluric acid by reduction of dimethyluric acid glycol with zinc and acetic acid.



Electrolytic Reduction. In 1901 Tafel commented on the fundamental importance to the chemistry of uric acid and the xanthines of the indirect reduction of the ketopurines, which was accomplished by replacing the oxygen atoms by chlorine and then the chlorine by hydrogen. He called attention to the observation of Strecker²⁴² that direct reduction of uric acid by heating with hydriodic acid resulted in the splitting of the purine molecule with the formation of ammonia, carbon dioxide, and glycooll; and concluded his discussion of the work on uric acid

²³⁸ Johnson and Joyce, *J. Am. Chem. Soc.*, **38**, 1385 (1916).

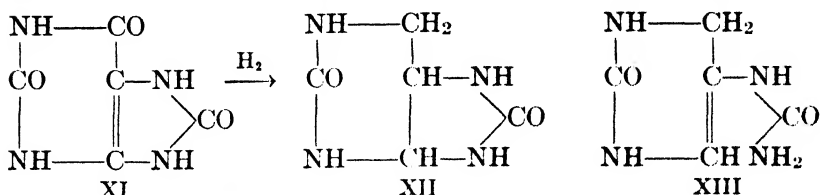
²³⁹ Johnson and Joyce, *ibid.*, **38**, 1854 (1916).

²⁴⁰ Biltz and Heyn, *Ber.*, **45**, 1666 (1912).

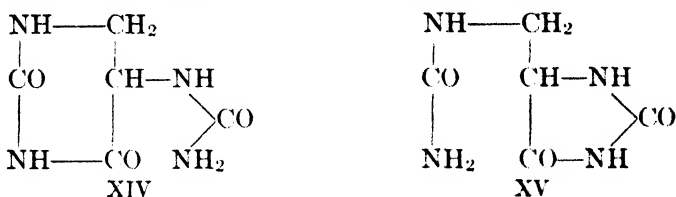
²⁴¹ Biltz, *Ann.*, **433**, 137 (1923).

²⁴² Strecker, *Z. Chem.*, **11**, 215 (1868).

reduction previous to his own by citing Fischer's²⁴³ inability to confirm an earlier statement of Strecker²⁴⁴ that uric acid was reduced by sodium amalgam to xanthine and sarkine (hypoxanthine). Tafel²⁴⁵ reported the direct reduction, electrolytically, with lead cathode in sulfuric acid solution, of uric acid (XI) to purone (XII). If the temperature of the reduction is not kept low (5-8°), isopurone is also formed. Purone, itself, when warmed in alkaline or alcoholic sulfuric acid solution undergoes isomerization to isopurone, to which Tafel²⁴⁶ assigned structure

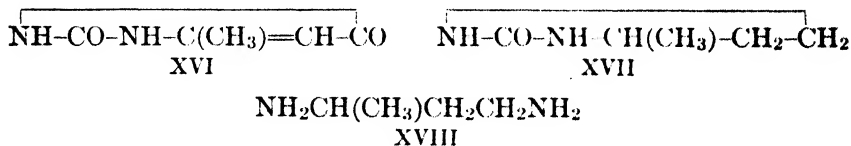


XIII. If the reduction is carried out slowly and in a high concentration of sulfuric acid, a tetrahydrouic acid is also formed. Structures XIV



or XV were assigned²⁴⁷ to this tetrahydrouic acid, primarily because it gives α,β -diaminopropionic acid on hydrolysis with barium hydroxide. Several methyluric acids give methylpurones²⁴⁸ on similar electrolytic treatment.

On electrolytic reduction, 6-methyluracil (XVI) gives 2-keto-6-methylhexahydropyrimidine (XVII) and 1,3-diaminobutane (XVIII)²⁴⁹.



²⁴³ Fischer, *Ber.*, **17**, 329 (1884).

²⁴⁴ Strecker, *Ann.*, **131**, 121 (1864).

²⁴⁵ Tafel, *Ber.*, **34**, 258 (1901).

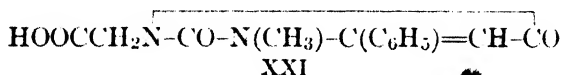
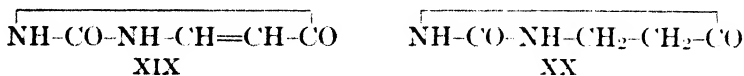
²⁴⁶ Tafel and Houseman, *Ber.*, **40**, 3743 (1907).

²⁴⁷ Tafel, *Ber.*, **34**, 1181 (1901).

²⁴⁸ Tafel, *Ber.*, **30**, 279 (1901).

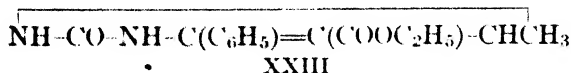
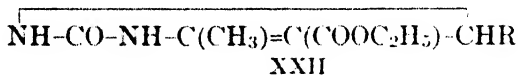
²⁴⁹ Tafel and Weinschenk, *Ber.*, **33**, 3378 (1900).

Catalytic Hydrogenation. Apparently, the first investigators to apply catalytic hydrogenation to the pyrimidine series were Levene and LaForge.^{250, 251} They hydrogenated the nucleoside uridine to dihydrouridine by Paal's method, using colloidal palladium. Johnson and Brown²⁵² recorded the next observation on pyrimidine hydrogenation. They found that with a colloidal palladium or platinum catalyst, the 5,6-double bond of uracil (XIX) is quite resistant to reduction at 20-25°, but at 75° is reduced to dihydrouracil (XX). The reduction of the 5,6-double bond of 1-methyl-6-phenyl-3-uracilacetic acid (XXI) and its methyl ester could not be accomplished by Evans and Johnson²⁵³ using



a palladium catalyst at 25°. The rate of hydrogenation²⁵⁴ of the cyclic double bond of carboxyuracil and 1-methyluracil is very slow with a platinum catalyst at 25°. Folkers and Johnson²⁵⁵ studied the reduction with a platinum catalyst of some 2-keto-1,2,3,4-tetrahydropyrimidines (XXII) where R was the phenyl, cyclohexyl, methyl, styryl, or phenylethyl group. In all cases, the pyrimidine double bond resisted hydrogenation, but the 4-aryl groups were readily hydrogenated. However, an isomer of one of these pyrimidines (XXIII) did yield a cyclohexylhexahydropyrimidine by the same technique.

Folkers and Johnson²⁵⁶ reported the satisfactory hydrogenation over a nickel catalyst at the 5,6-double bond of pyrimidines of structure XXII at 175° and under 200 atmospheres pressure, and later described²⁵⁷ the



²⁵⁰ Levene and La Forge, *Ber.*, **45**, 619 (1912).

²⁵¹ Levene, *J. Biol. Chem.*, **63**, 653 (1925).

²⁵² Johnson and Brown, *Proc. Natl. Acad. Sci. U. S.*, **7**, 75 (1921); Brown and Johnson, *J. Am. Chem. Soc.*, **45**, 2702 (1923).

²⁵³ Evans and Johnson, *ibid.*, **52**, 5000 (1930).

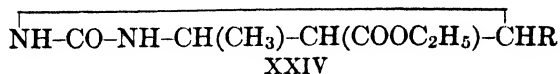
²⁵⁴ Hilbert, *ibid.*, **54**, 2078 (1932).

²⁵⁵ Folkers and Johnson, *ibid.*, **55**, 1140 (1933).

²⁵⁶ Folkers and Johnson, *ibid.*, **55**, 2886 (1933).

²⁵⁷ Folkers and Johnson, *ibid.*, **56**, 1180 (1934).

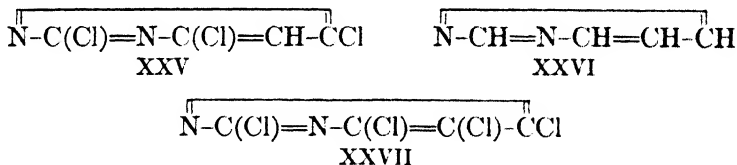
hydrogenation of tetrahydropyrimidines to isomeric hexahydropyrimidines (XXIV), by the use of a copper-barium-chromium oxide catalyst under elevated temperatures and pressures.



Miscellaneous Pyrimidine and Purine Reduction Experiments

Reduction reactions which replace by hydrogen an atom directly attached to one of the atoms in the pyrimidine or purine nucleus are placed in this group. The most outstanding example is the reduction of chloropyrimidines and chloropurines to hydrogen derivatives. Such reactions are definitely characteristic of the nucleus. Those cases should be grouped separately in which the reduction is almost entirely a consideration of the specific group, as for example, the reduction to uramil of nitrobarbituric acid²⁵⁸ or alloxan-phenylhydrazone²⁵⁹ by tin and hydrochloric acid, or nitrosobarbituric acid²⁶⁰ by hydrogen iodide to 5-aminobarbituric acid; and the reduction of 5-nitrouracil²⁶¹ to 5-aminouracil and isobarbituric acid by zinc and acid.

Reductions by Sodium Amalgam, Hydriodic Acid, Zinc Chloride, Zinc and Acids, etc. Pyrimidine (XXVI) is prepared by the action of zinc dust and water on 2,4,6-trichloropyrimidine²⁶² (XXV) and 2,4,5,6-tetrachloropyrimidine²⁶³ (XXVII).



Wheeler²⁶⁴ observed that 2,4-dichloropyrimidine (XXVIII) is reduced by hydriodic acid and phosphorus to 4-ketopyrimidine (XXIX). The more reactive 4-chlorine atom is hydrolyzed, whereas the 2-chlorine



²⁵⁸ Hartman and Sheppard, "Organic Syntheses," John Wiley & Sons, New York City (1932), Vol. XII, p. 84.

²⁵⁹ Kühling, *Ber.*, **31**, 1973 (1898).

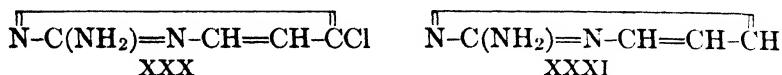
²⁶⁰ Baeyer, *Ann.*, **127**, 223 (1863).

²⁶¹ Behrend and Roosen, *Ann.*, **251**, 235 (1889).

²⁶² Gabriel, *Ber.*, **33**, 3667 (1900).

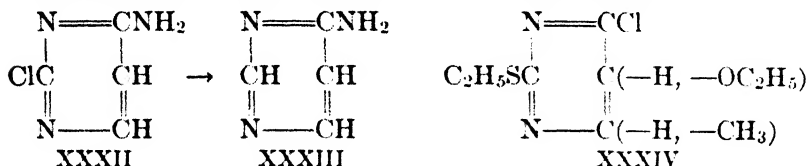
²⁶³ Emery, *Ber.*, **34**, 4180 (1901).

atom is reduced. 2-Amino-4-chloropyrimidine (XXX) is reduced to 2-aminopyrimidine (XXXI) by zinc dust, but this reagent does

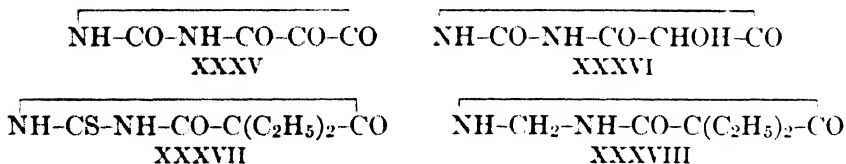


not reduce the isomeric 2-chloro-4-aminopyrimidine (XXXII). However, the pyrimidine XXXII is reduced to the 4-aminopyrimidine (XXXIII) by means of hydriodic acid. Obviously, a 2-chlorine atom in this series is not reduced by means of zinc. Johnson and Joyce²⁶⁵ reported the reduction of 2,4-dichloro-5-ethoxypyrimidine by zinc dust to 2-chloro-5-ethoxypyrimidine.

Mercaptochloropyrimidines,²⁶⁵ such as represented by structure XXXIV, and the corresponding 2-anilino-²⁶⁶ and 2-methylamino-²⁶⁷ derivatives are reduced by zinc dust to the corresponding mercapto- and



aminopyrimidines respectively. Alloxan²⁶⁸ (XXXV) is reduced to dialuric acid (XXXVI) by the use of strongly acidic zinc chloride solution. Dimethylalloxantine is reduced to methylalloxantine by sodium amalgam. Diethylthiobarbituric acid (XXXVII) is reduced by sodium amalgam to desoxyveronal (XXXVIII).²⁷⁰



Fichter and Stenzl²⁷¹ were able to reduce 5-isopropylbarbituric acid to 5-isopropyluracil, and 5-ethylbarbituric acid to a mixture of 5-ethyluracil and 5-ethyldihydrouracil, by means of a lead-sodium alloy and acids.

²⁶⁴ Wheeler, *J. Biol. Chem.*, **3**, 289 (1907).

²⁶⁵ Johnson and Joyce, *J. Am. Chem. Soc.*, **37**, 2151 (1915).

²⁶⁶ Johnson and Heyl, *Am. Chem. J.*, **38**, 237 (1907).

²⁶⁷ Johnson and Mackenzie, *ibid.*, **42**, 355 (1909).

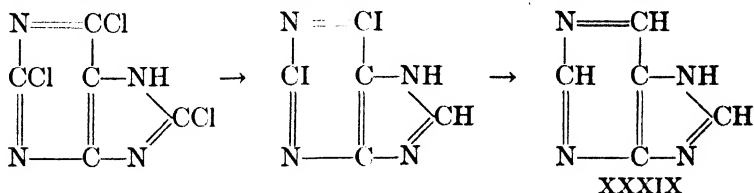
²⁶⁸ Fischer, *Ann.*, **215**, 258 (1882); Baeyer, *Ann.*, **127**, 13 (1863).

²⁶⁹ Biltz and Damm, *Ber.*, **46**, 3662 (1913).

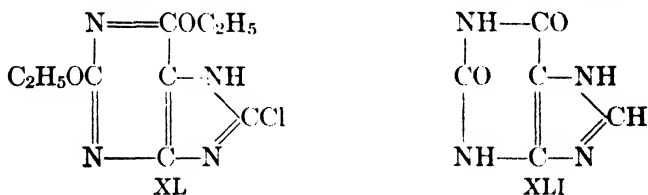
²⁷⁰ Einhorn, *Ann.*, **359**, 176 (1908).

²⁷¹ Fichter and Stenzl, *Helv. Chim. Acta*, **17**, 665 (1934).

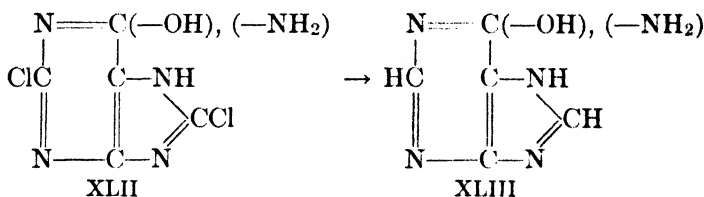
Purine (XXXIX) was first prepared by E. Fischer²⁷² in 1899 by converting 2,6,8-trichloropurine by hydriodic acid and phosphonium iodide at 0° to 2,6-diiodopurine, and this to purine by zinc dust and water. Similarly, 2-iodo-7-methylpurine, and 2-iodo-9-phenylpurine



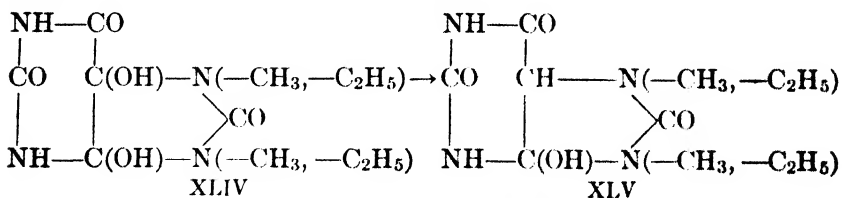
are reduced to 7-methyl- and 9-phenylpurine, respectively. The reduction takes place smoothly with all halogenopurines, and also with those derivatives which contain (beside the halogen), oxygen, sulfur, or N-methyl or amino groups. For example, 2,6-diethoxy-8-chloropurine (XL) gives xanthine (XLI).



(XLII) gives hypoxanthine and adenine²⁷³ (XLIII) respectively.



7,9-Dimethyl- and 7,9-diethyluric acid glycols (XLIV) give 7,9-dimethyl- and 7,9-diethyl-4-hydroxy-4,5-dihydrouric acids (XLV) when

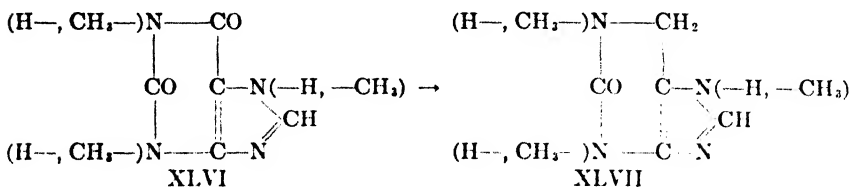


²⁷² Fischer, *Ber.*, **31**, 2550, 2564 (1898); **32**, 493 (1899).

²⁷³ Fischer, *Ber.*, **30**, 2235, 2240 (1897).

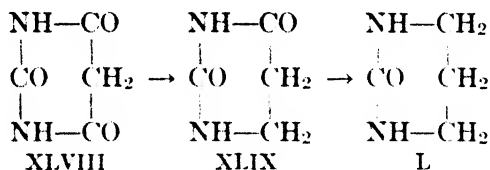
heated with phosphorus tribromide.²⁴¹ Theophylline and paraxanthine are obtained in yields of 80 per cent by heating the 8-chloro derivatives in tetrahydronaphthalene with less than one atom of iodine.²⁷⁴

Electrolytic Reduction. The general reduction equation of Tafel's electrolytic reductions of xanthines to desoxyxanthines in sulfuric acid solution with a lead cathode may be represented by structures XLVI and XLVII. Xanthine,²⁷⁵ 3-methylxanthine,²⁷⁶ 1-methylxanthine,²⁷⁷



heteroxanthine,²⁷⁶ theobromine,²⁷⁸ theophylline,²⁷⁹ paraxanthine,²⁸⁰ and caffeine²⁸⁰ are all reduced to the corresponding desoxyxanthine derivatives. Guanine²⁸¹ gives desoxyguanine.

In the pyrimidine series, Tafel reduced electrolytically, barbituric acid,²⁸² (XLVIII) to dihydrouracil (XLIX) and at a higher temperature to 2-ketohexahydropyrimidine (L). Ethylbarbituric acid²⁸³ (LI)



gives 5-ethyldihydrouracil (LII), whereas veronal (LIII) undergoes reduction of the 2-keto group, giving desoxyveronal (LIV). Dialuric acid, uramil, and alloxan give principally dihydrouracil on reduction.²⁸⁴ Dialuric acid also gives 2-ketohexahydropyrimidine and 2-keto-5-hydroxyhexahydropyrimidine.

²⁷⁴ Ger. pat. 576,604, May 12, 1933; [*C. A.*, **27**, 5737 (1933)].

²⁷⁵ Tafel and Ach., *Ber.*, **34**, 1165 (1901).

²⁷⁶ Tafel and Weinschenk, *Ber.*, **33**, 3369 (1900).

²⁷⁷ Tafel and Herterich, *Ber.*, **44**, 1033 (1911).

²⁷⁸ Tafel, *Ber.*, **33**, 3194 (1899).

²⁷⁹ Tafel and Dodt, *Ber.*, **40**, 3752 (1907).

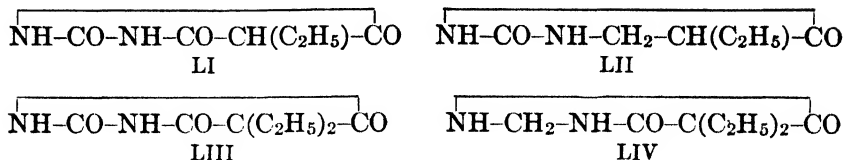
²⁸⁰ Baillie and Tafel, *Ber.*, **33**, 3206 (1899).

²⁸¹ Tafel and Ach., *Ber.*, **34**, 1170 (1901).

²⁸² Tafel and Weinschenk, *Ber.*, **33**, 3383 (1900); Tafel, *Ber.*, **34**, 144 (1901); cf. Weidel and Roithner, *Monatsh.*, **17**, 175 (1896).

²⁸³ Tafel and Thompson, *Ber.*, **40**, 4489 (1907).

²⁸⁴ Tafel and Reindl, *Ber.*, **34**, 3286 (1901).



Catalytic Hydrogenation. A palladium catalyst has been used by Rosenmund and Zetzsche²⁸⁵ to transform 8-chlorocaffeine to caffeine. A patent²⁸⁶ describes the hydrogenation in the presence of a catalyst of 8-chlorotheophylline and 8-chloroparaxanthine in aqueous alkaline solution at 100° and 30-40 atmospheres pressure to theophylline and paraxanthine.

NATURAL OCCURRENCE OF PYRIMIDINES AND PURINES

Pyrimidines

Certain representatives of the pyrimidine group have been discovered in nature occurring as normal constituents of the nucleic acids. These derivatives are keto forms of pyrimidine and are classified as cyclic ureides containing —CO—NH— groupings. The derivatives thus far discovered in nature are represented structurally below. According to the results obtained by recent investigators in the newer field of vitamin chemistry, it has been established that a pyrimidine cycle actually occurs in vitamin B₂ (C₁₇H₂₀O₆N₄), and also in vitamin B₁. A summary of the present conclusions regarding the constitutions of both vitamins B₁ and B₂ is given later in this chapter.

The pyrimidine compounds taking part in the organized structure of natural nucleic acids include uracil,²⁸⁷ thymine²⁸⁸ (5-methyluracil), and cytosine.²⁸⁹

²⁸⁵ Rosenmund and Zetzsche, *Ber.*, **51**, 582 (1918).

²⁸⁶ Boehringer & Son, Ger. pat., 582,435; [*C. A.*, **27**, 5754 (1933)].

²⁸⁷ *Discovery*: Ascoli, *Z. physiol. Chem.*, **31**, 161 (1900); Kossel and Steudel, *ibid.*, **37**, 245 (1902); Levene, *ibid.*, **39**, 4 (1903); Levene and Stookey, *ibid.*, **41**, 404 (1904); Levene, *ibid.*, **37**, 527 (1903); Reh, *Beitr. Chem. Physiol. Path.*, **3**, 569 (1903); Jones, *Z. physiol. Chem.*, **42**, 35 (1904); Levene and Mandel, *ibid.*, **49**, 262 (1906); Engeland and Kutscher, *Zentr. Physiol.*, **24**, 589 (1910).

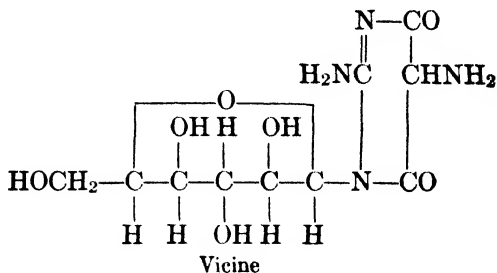
Synthesis: Fischer and Roeder, *Ber.*, **34**, 3751 (1901); Wheeler and Merriam, *Am. Chem. J.*, **29**, 478 (1903); Gabriel and Colman, *Ber.*, **36**, 3379 (1903); Davidson and Baudisch, *J. Am. Chem. Soc.*, **48**, 2379 (1926); Büttner, *Ber.*, **36**, 2227 (1903); Gabriel, *Ber.*, **38**, 1690 (1905).

²⁸⁸ *Discovery*: Kossel and Neumann, *Ber.*, **26**, 2753 (1893); Miescher and Schmiedeberg, *Arch. expil. Path. Pharmacol.*, **37**, 100 (1896); Jones, *Z. physiol. Chem.*, **29**, 20 (1900); Steudel, *ibid.*, **30**, 539 (1900); **32**, 241 (1901).

Synthesis: Fischer and Roeder, *loc. cit.*; Wheeler and Merriam, *loc. cit.*; Wheeler and McFarland, *Am. Chem. J.*, **43**, 19 (1910); Gerngross, *Ber.*, **38**, 3408 (1905).

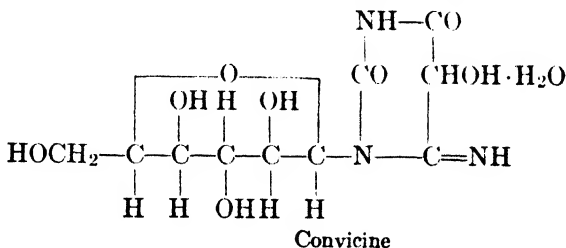
²⁸⁹ *Discovery*: Kossel and Neumann, *Ber.*, **27**, 2215 (1894); Kossel and Steudel, *Z.*

present chemical evidence may be assigned the constitutional formula given below.²⁹¹



It was first isolated by Ritthausen²⁹² from vetch seeds.

The glucoside convicine was also isolated from vetch seeds by Ritt-hausen, and he assigned to it the empirical formula $\text{C}_{10}\text{H}_{15}\text{N}_3\text{O}_8 \cdot \text{H}_2\text{O}$. It is characterized by its behavior on hydrolysis when it yields alloxantine. Schulze and Trier²⁹³ proposed a formula corresponding to alloxantine diglucoside plus two molecules of ammonia. Johnson²⁹⁴ suggested that the formula $\text{C}_{10}\text{H}_{15}\text{N}_3\text{O}_8$ corresponded to that of either an aminoglycoside of dialuric acid or a glucoside of uramil. Fisher and Johnson²⁹⁵ repeated successfully the original work of Ritthausen and studied the behavior of convicine on acid hydrolysis and on oxidation.



²⁹¹ Levene, *J. Biol. Chem.*, **18**, 305 (1914).

²⁹² Ritthausen and Kruesler, *J. prakt. Chem.*, [2] **3**, 333, (1870); Ritthausen "Die Eiweisskörper der Getreidearten," Cohen and Son, Bonn (1872), pp. 168-169; *J. prakt. Chem.*, [2] **7**, 374 (1873); *Ber.*, **9**, 301 (1876); *J. prakt. Chem.*, [2] **24**, 202 (1881); **29**, 359 (1884); Schulze, *Z. physiol. Chem.*, **15**, 140 (1891); *Ber.*, **22**, 1827 (1889), *Z. physiol. Chem.*, **17**, 193 (1893); Ritthausen, *Ber.*, **29**, 894, 2108 (1896); v. Lippman, *Ber.*, **29**, 2653 (1896); Ritthausen, *J. prakt. Chem.*, [2] **59**, 480, 482 (1899); Ritthausen and Prouss, *ibid.*, [2] **59**, 487 (1899); Winterstein, *Z. physiol. Chem.*, **105**, 258 (1919); Schulze and Trier, *ibid.*, **70**, 150 (1910-11); Johnson, *J. Am. Chem. Soc.*, **36**, 337 (1914); Johnson and Johns, *ibid.*, **36**, 545 (1914); Fischer, *Ber.*, **47**, 2611 (1914); Levene, *J. Biol. Chem.*, **18**, 305 (1914); Levene and Senior, *ibid.*, **25**, 607 (1916); Herissey and Cheymol, *Compt. rend.*, **191**, 387 (1930); *Bull. soc. chim. biol.*, **13**, 29 (1931); Fisher and Johnson, *J. Am. Chem. Soc.*, **54**, 2038 (1932).

²⁹³ Schulze and Trier, *J. physiol. Chem.*, **70**, 150 (1910-11).

²⁹⁴ Johnson, *J. Am. Chem. Soc.*, **36**, 337 (1914).

²⁹⁵ Fisher and Johnson, *ibid.*, **54**, 2038 (1932).

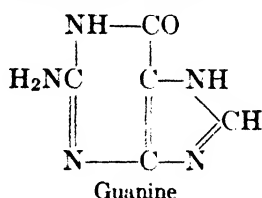
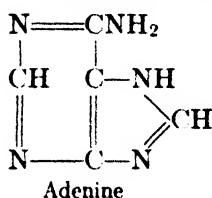
As a result of their investigation they proposed the formula below for this glucoside. The pyrimidine nucleus of such a glucoside—*isouramil*—has been synthesized recently by Bogert and Davidson.²⁹⁶

Purines

Purine compounds are found widely distributed throughout the animal and plant kingdoms. Several of them, for example, uric acid, have been shown to be important end products of metabolic changes. Not only do they occur in nature in a free state, but they have also been found to exist in the form of glycosides.

Only two representatives of the purine group have thus far been observed to enter into the structure of the nucleic acid molecule. These are adenine²⁹⁷ and guanine.²⁹⁸ The constitutions of these two naturally occurring purines are expressed below.

PURINES OCCURRING IN NUCLEIC ACIDS



The most widely distributed purine substances found in nature outside of the two occurring in nucleic acids are xanthine²⁹⁹ and hypoxanthine.³⁰⁰ These are possibly formed by deamination of guanine and adenine. Certain methylated derivatives of xanthine which are classed among alkaloidal substances, namely, caffeine,³⁰¹ theobromine,³⁰²

²⁹⁶ Bogert and Davidson, *Proc. Natl. Acad. Sci. U. S.*, **18**, 490 (1932).

²⁹⁷ *Discovery*: Kossel, *Ber.*, **18**, 79, 1928 (1885); *Z. physiol. Chem.*, **10**, 250 (1886); **12**, 241 (1888).

²⁹⁸ *Discovery*: Unger, *Pogg. Ann.*, **65**, 222 (1845); Unger, *Ann.*, **58**, 18 (1846); Strecker, *Ann.*, **108**, 141 (1858).

²⁹⁹ *Discovery*: Marcet, "An Essay on the Chemical History and Medical Treatments of Calcal Disorders," London (1817); Scherer, *Ann.*, **113**, 257 (1859); Wöhler and Liebig, *Ann.*, **26**, 340 (1838); Krüger and Salomon, *Z. physiol. Chem.*, **24**, 371 (1898); Strecker, *Ann.*, **118**, 157 (1861); Krüger and Schittenhelm, *Z. physiol. Chem.*, **35**, 161 (1902); Schreiner and Shorey, *J. Biol. Chem.*, **8**, 385 (1910).

³⁰⁰ *Discovery*: Scherer, *Ann.*, **73**, 328 (1850); For chemistry of sarkin, see Strecker, *Ann.*, **108**, 129, 141 (1858); Krüger, *Z. physiol. Chem.*, **18**, 423, 459 (1894).

³⁰¹ *Discovery*: Rungor, *Phytochem. Entdeckungen*, 144 (1820); Seguin, *Ann. chim. phys.*, **92**, 1 (1814); Brugnatelli, *ibid.*, **95**, 299 (1815); Oudry, *Mag. Pharm.*, **19**, 49 (1827); Günther, *J. prakt. Chem.*, **10**, 273 (1837); Mulder, *Pogg. Ann.*, **43**, 161 (1838); *Ann.*, **28**, 319 (1838); Jobst, *Ann.*, **25**, 63 (1838); Martius, *Ann.*, **36**, 93 (1840); Berthemot and Dechastelus, *J. pharm. Trans.*, **26**, 514; *Ann.*, **36**, 90 (1840); Berzelius, *Jahrb. Chem.*, **21**, 322 (1842); Attfield, *Pharm. J.*, [2] **6**, 457 (1865); Schmidt, *Ann.*, **217**, 306 (1883).

³⁰² *Discovery*: Woskressensky, *J. prakt. Chem.*, **23**, 394 (1841); *Ann.*, **41**, 125 (1842);

and theophylline,³⁰³ are also found in the plant kingdom. Also as products of metabolic change the following alkylated purines have been found: 1,7-dimethylxanthine,³⁰⁴ 7-methylxanthine,³⁰⁵ 1-methylxanthine,³⁰⁶ and 3-methylxanthine.³⁰⁷

Glycosidic Combinations of Purines and Pyrimidines

An important group of substances coming under this heading are the glycosides known as ribosides. Thus far, pyrimidine ribosides³⁰⁸ have not been found in a free state, and result only as degradation products of nucleic acids. Some purine ribosides, which have been shown to occur in a free state, are the following: guanosine (or as this was first called, vernine),³⁰⁹ inosine,³¹⁰ and xanthosine.³¹¹ The glycoside adenosine³¹² is the precursor of inosine and was first obtained in a free state by hydrolysis of yeast nucleic acid.

No pyrimidine or purine glycosides of the desoxyriboside type³¹³

Heckel and Schlagdenhaufen, *J. pharm. chim.*, [5] **8**, 177 (1883); Decker, *Just's botan. Jahresber.*, **2**, 14 (1902); Hilger, *Apoth. Ztg.*, **7**, 469 (1892).

³⁰³ *Discovery*: Kossel, *Z. physiol. Chem.*, **13**, 298 (1889).

³⁰⁴ *Discovery*: (in urine): Salomon, *ibid.*, **11**, 415 (1887); *Ann. Chem. Med.*, **1**, 163 (1879); Thudichum, *Compt. rend.*, **106**, 1805 (1888); Salomon, *Arch. Physiol.* (duBois-Reymond), 426 (1882); *Ber.*, **16**, 195 (1883); **18**, 3406 (1885); *Z. klin. Med. Suppl.*, **7**, 63 (1884); *Virchow's Arch. path. Anat.*, **125**, 554 (1891).

³⁰⁵ *Discovery*: (in urine): Krüger and Salomon, *Z. physiol. Chem.*, **24**, 364 (1898); **26**, 350 (1898-99); Salomon, *ibid.*, **11**, 413 (1887); Salkowski's *Festschr.*, Berlin (1904); Krüger and Schmidt, *Ber.*, **32**, 2677 (1899); Bondzynski and Gottlieb, *Ber.*, **28**, 1113 (1895); *Arch. expil. Path. Pharmacol.*, **36**, 45 (1895).

³⁰⁶ *Discovery*: (in urine): Krüger and Salomon, *Z. physiol. Chem.*, **24**, 364 (1898); **26**, 358 (1898-99); Krüger and Schmidt, *Ber.*, **32**, 2680 (1899); Krüger, *Ber.*, **32**, 3336 (1899); Okerblom, *Z. physiol. Chem.*, **28**, 60 (1899); Engelmann, *Ber.*, **42**, 177 (1909); Krüger, *Ber.*, **33**, 3665 (1900).

³⁰⁷ *Discovery*: (in urine): Schmidt, Dissertation, Berlin, 1904; Krüger and Schmidt, *Ber.*, **32**, 2680 (1899); Krüger and Schmidt, *Z. physiol. Chem.*, **36**, 1 (1902); Albanese, *Ber.*, **32**, 2280 (1899); Krüger and Schmidt, *Arch. expil. Path. Pharmacol.*, **45**, 259 (1901).

³⁰⁸ *Uridine and Cytidine*:—Levene and LaForge, *Ber.*, **45**, 608 (1912); Emerson and Cerecedo, *J. Biol. Chem.*, **87**, 453 (1930); Levene and Simms, *ibid.*, **65**, 519 (1925); **70**, 327 (1926); Levene, Bass, and Simms, *ibid.*, **70**, 229 (1926); Levene and Jacobs, *Ber.*, **43**, 3150 (1910).

³⁰⁹ Schulze and Bosshard, *Z. physiol. Chem.*, **9**, 420, 443 (1885); **10**, 80 (1886); Schulze and Castoro, *ibid.*, **41**, 455 (1904); Schulze, *ibid.*, **66**, 128 (1910); Schulze and Trier, *ibid.*, **70**, 143 (1910); Schulze and v. Planta, *ibid.*, **10**, 326 (1886); Schulze, *J. prakt. Chem.*, [2] **32**, 433 (1885); Levene and Jacobs, *Ber.*, **42**, 2474 (1909); **43**, 3163 (1910).

³¹⁰ Hauser and Wenzel, *Monatsh.*, **29**, 157 (1908); Levene and Jacobs, *Ber.*, **42**, 335, 2474 (1909); *Ber.*, **42**, 2703 (1909).

³¹¹ Levene and Jacobs, *Ber.*, **43**, 3163 (1910); see also Davis, Newton, and Benedict, *J. Biol. Chem.*, **54**, 595 (1922); Levene, *ibid.*, **55**, 437 (1923).

³¹² Levene and Jacobs, *Ber.*, **42**, 2703 (1909).

³¹³ Feulgen, "Chemie und Physiologie der Nukleinstoffe," Bornträger, Berlin (1923).

have been isolated in a free state. They have been found, thus far, only as degradation products of a nucleic acid.³¹⁴

Two pyrimidine glycosides of the hexoside type have been found to occur in plants, namely, vicine and convicine, which have already been referred to in a preceding paragraph. An interesting glycoside of another type is adenine methylthiopentoside which is obtained from yeast extracts.³¹⁵

NUCLEOSIDES, MONONUCLEOTIDES, AND POLYNUCLEOTIDES (NUCLEIC ACIDS)

Purines and pyrimidines occur in plants and in animal tissues either in the free state or as constituents of more complex substances. Of these latter the best known are nucleosides, mononucleotides, and polynucleotides or nucleic acids.

Nucleosides

Nucleosides are glycosides, whose aglucons may be either a purine or a pyrimidine base. The carbohydrate (pp. 1399, 1477) components of those now known are *d*-glucose, *d*-ribose, *d*-2-desoxyribose, and thio-methylpentose. The aglucons thus far discovered are adenine, guanine, cytosine, uracil, thymine, methylcytosine, and 2,5-diamino-4,6-dioxy-pyrimidine. Only one derivative of glucose is known, vicine, and its aglucon is 2,5-diamino-4,6-dioxypyrimidine.³¹⁶ One nucleoside is a derivative of a thiomethylpentose but the structure of this thiomethylpentose is as yet not known. All other known nucleosides are derivatives of *d*-ribose or of 2-desoxy-*d*-ribose.

The details of the structure of the nucleosides is determined by the position of the sugar residue on the base and by the size of the ring of the sugar residue. All the *d*-ribose and *d*-desoxyribose derivatives have been found to have the furanoside structure. The ring structure was identified either by complete methylation of nucleosides with subsequent hydrolysis and oxidation of the methylated ribose to dimethyltartaric acid³¹⁷ or by the formation of a triphenylmethyl derivative. Primary alcoholic groups combine preferentially with triphenylmethyl chloride; the formation of a

³¹⁴ Levene and London, *J. Biol. Chem.*, **81**, 711 (1929); **83**, 793 (1929); Levene and Mori, *ibid.*, **83**, 803 (1929); Levene, Mikeska, and Mori, *ibid.*, **85**, 785 (1930); Bergmann, Schotte, and Lechinsky, *Ber.*, **55**, 158 (1922).

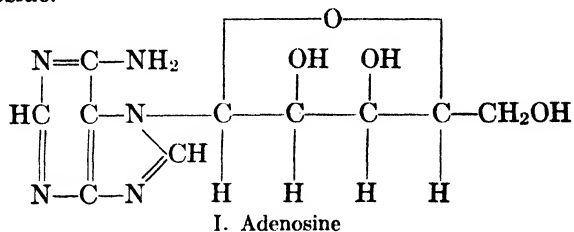
³¹⁵ Mandel and Dunham, *J. Biol. Chem.*, **11**, 85 (1912); Suzuki, *J. Tokyo Chem. Soc.*, **34**, 1134 (1914); Suzuki, Odake, and Mori, *J. Agr. Chem. Soc. (Japan)*, **1**, No. 2, (1924); *Biochem. Z.*, **154**, 278 (1924); Levene, *J. Biol. Chem.*, **59**, 465 (1924); Levene and Sobotka, *ibid.*, **65**, 551 (1925); Sobotka, *ibid.*, **69**, 267 (1926).

³¹⁶ Levene and Senior, *J. Biol. Chem.*, **25**, 607 (1916).

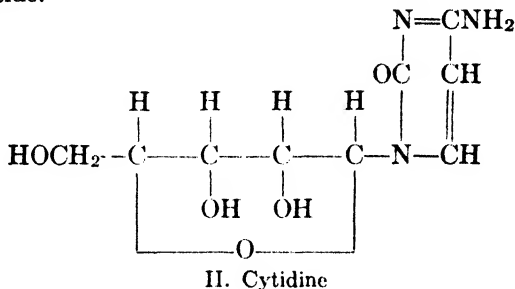
³¹⁷ Levene and Tipson, *ibid.*, **94**, 809 (1932).

trityl derivative indicates that the primary alcoholic group of the pentose is not involved in the ring formation.³¹⁸

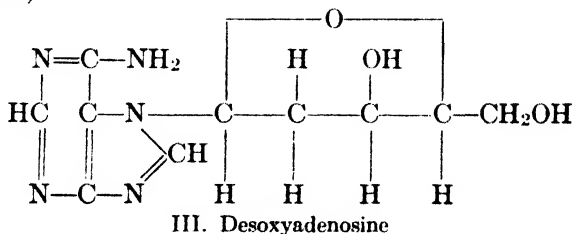
A typical purine nucleoside is adenosine (I), which is 7-adenine *d*-ribofuranoside.



A typical pyrimidine nucleoside is cytidine (II), which is 3-cytosine *d*-ribofuranoside.



A typical desoxyribose nucleoside is 7-adenine *d*-desoxyribofuranoside. (III)



Desoxyribose, like ribose, was discovered in nature for the first time as a component of a nucleoside. This sugar is so unstable that only by very gentle treatment can it be separated from the aglucon without destruction. Its ready transformation into levulinic acid was a surprising observation.

The first nucleoside discovered in nature was guanosine.³¹⁹ Its

³¹⁸ Bredereck, *Ber.*, **66**, 198 (1933).

³¹⁹ Schulze and Boshard, *Z. physiol. Chem.*, **9**, 443 (1885); **10**, 80 (1886).

identity was then not fully known and it was described under the name "vernine." It was later found to be identical with guanosine. The other nucleoside occurring in nature in free state is adenine thiomethylpentoside.

The nucleosides now known are adenosine, guanosine, cytidine, uridine, desoxyadenosine, desoxyguanosine, desoxycytidine, desoxyuridine, thymidine (3-thymine desoxyribofuranoside), vicine, and adenine thiomethylpentoside. All except the latter two are accessible through cleavage of nucleic acids. (Guanosine is found also in tissue extracts.) The *ribose* nucleosides are prepared by heating the ribonucleic acids under pressure on the slightly alkaline side. The desoxyribosides could not be obtained from the desoxyribonucleic acids by purely chemical means and enzymatic cleavage had to be resorted to.³²⁰

Properties. All nucleosides are crystalline substances, soluble in water and insoluble in most organic solvents. The purine ribosides are readily hydrolysable with dilute mineral acids, yielding the aglucon and *d*-ribose. Most of the pyrimidine nucleosides are very resistant to acid hydrolysis. When stronger acid (10 per cent hydrochloric acid) is used, the sugar component is destroyed and only the aglucon can then be identified. An exception is vicine which behaves like a purine nucleoside. However, when the aglucon is catalytically hydrogenated, then the hydrogenated pyrimidine nucleoside behaves toward acid as an ordinary glycoside. The purine nucleosides can readily be differentiated from the pyrimidine nucleosides by the orcinol test, the former giving the usual pentose test whereas the latter give only a faint pink coloration.

Two nucleosides having the sugar residue attached to a nitrogen atom of the aglucon have thus far been prepared synthetically, namely, 7-adenine-*d*-glucopyranoside³²¹ and 3-uracil-*d*-glucopyranoside.³²² Several nucleosides having the sugar residue attached to the oxygen atom have been prepared.³²³

Mononucleotides

Nucleotides are phosphoric esters of nucleosides. Whereas few nucleosides have been discovered in nature, several nucleotides are known to occur in animal and plant tissues and to some of them important biological functions are attributed.

Depending upon the position of the phosphoric acid residue, there are

³²⁰ Levene and London, *J. Biol. Chem.*, **81**, 711 (1929); **83**, 793 (1929).

³²¹ Fischer and Helferich, *Ber.*, **47**, 210 (1914).

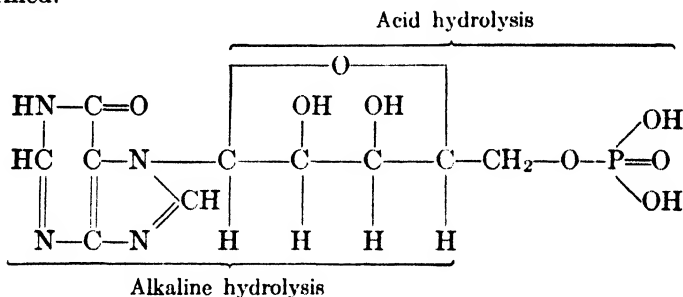
³²² Hilbert and Johnson, *J. Am. Chem. Soc.*, **52**, 4489 (1930).

³²³ Levene and Sobotka, *J. Biol. Chem.*, **65**, 463 (1925).

theoretically three isomeric nucleotides for the ribose nucleotides and two for the desoxyribonucleotides. In fact, among the ribose nucleotides two types have been discovered differing in the position of the phosphoric acid residue. Those of one type have the phosphoric acid residue in position 5 of the sugar, the others in position 3.

5-Phosphoribofuranosides. Nucleotides of the first type occur in tissues in the free state and possess physiological action. A representative member of this group of substances is inosinic acid.

Inosinic Acid. This acid was obtained in crystalline form from beef extract by Liebig in 1847 but not until 1895 was it recognized by Hauser to be a derivative of phosphoric acid. Its structure was not cleared up until 1908–1911.³²⁴ It was also on that occasion that *d*-ribose was discovered to occur in a natural product and was the first time it was obtained in crystalline form. The structure of the substance emerges from the fact that on acid hydrolysis it yields a phosphoric ester of ribose and on neutral hydrolysis, inosine (hypoxanthine ribofuranoside) is formed.



The position of the phosphoric acid residue on carbon atom 5 was arrived at first on the basis of the fact that, on oxidation with nitric acid, phosphoribonic acid and not phosphoribotrihydroxyglutaric acid was obtained;³²⁴ further, by the rate of lactone formation in the phosphoribonic acid and finally, by the synthesis of 5-phosphoribose which had properties identical with that obtained on hydrolysis of inosinic acid.³²⁵

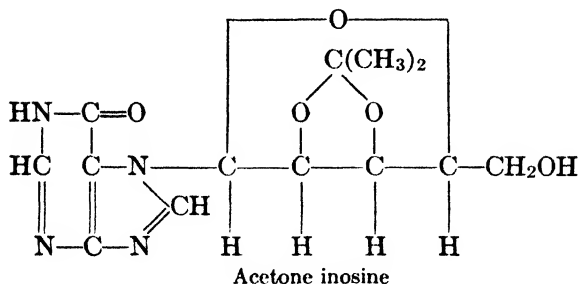
Additional proof of the position of the phosphoric ester residue was furnished by the "partial synthesis" of inosinic acid from acetone inosine, in which only position 5 of the ribose is not substituted.³²⁶

In fresh tissues, or fresh tissue extracts, inosine is present in only small traces, if at all. In its place is found its precursor, adenine 5-phosphori-

³²⁴ Levene and Jacobs, *Ber.*, **41**, 2703 (1908); **42**, 335, 1198 (1909); **44**, 746 (1911).

³²⁵ Levene and Stiller, *J. Biol. Chem.*, **104**, 299 (1934).

³²⁶ Levene and Tipson, *ibid.*, **111**, 313 (1935).

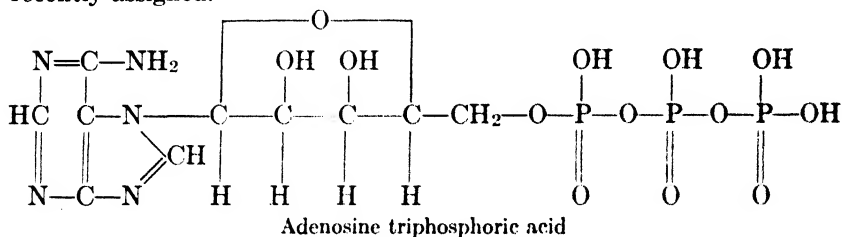


in crystalline form. Like the purine nucleotides, they are soluble in water and insoluble in most organic solvents but in distinction to purine nucleotides they possess great resistance toward hydrolytic agents.

Desoxyribosenucleotides. These substances have been described only recently.³²⁸ They have been obtained by enzymatic hydrolysis of desoxyribosenucleic acids. They have the general crystalline appearance of the ribonucleotides.

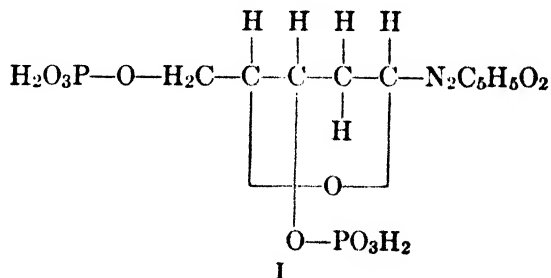
Nucleosidopolyphosphoric esters. Adenosinepolyphosphoric acids are biologically important substances playing a part in glucose utilization in animal and plant organisms, in alcoholic fermentation (p. 1525), etc.

To adenosinetriphosphoric acid the following structure has been recently assigned.



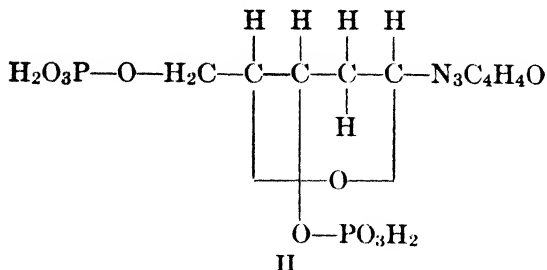
This substance was discovered in muscle extract by Lohmann³²⁹ and later by Fiske and Subbarow. By a specific enzyme it is converted into adenosine diphosphoric acid. Recently it was claimed that the latter nucleotide, in combination with a nicotinamide ribonucleotide, forms a dinucleotide which is the coenzyme in the process of conversion of glucose into lactic acid and of alcoholic fermentation.

Of great importance also are the diphosphoric esters of the pyrimidine desoxyribosides inasmuch as they form the key to the structure of the desoxyribonucleic acids. They are thymidinediphosphoric ester (I) and desoxycytidinediphosphoric ester (II).



³²⁸ Thannhauser and Ottensteen, *Z. physiol. Chem.*, **114**, 47 (1921); Klein, *ibid.*, **218**, 164 (1933); Klein and Thannhauser, *ibid.*, **218**, 173 (1933); **224**, 252 (1934).

³²⁹ Lohmann, *Naturwissenschaften*, **16**, 298 (1928); **17**, 624 (1929); Fiske and Subbarow, *Science*, **70**, 381 (1929).



These substances are characterized by their greater resistance toward hydrolytic agents but as soon as the bases are hydrogenated, both the base and the phosphoric acid residue are readily removed by mild treatment with mineral acids. Both nucleotides are obtained on hydrolysis of deoxyribosenucleic acid by boiling the acid with 2 per cent sulfuric acid.

Nucleic Acids (Polynucleotides)

In combination with proteins, histones, or protamines, nucleic acids are supposed to constitute the principal part of the nuclei of plant and animal cells; hence the name given to them by Altmann.³³⁰ The pioneer workers in the field of nucleic acids were Altmann, Miescher, and Kossel. As a result of their work the wide distribution of the substances in nature became known and also the identity of their nitrogenous components.

Nucleic acids are substances of very high molecular weight, the smallest unit having the properties of a nucleic acid being a tetranucleotide. On a few occasions, nucleic acids whose smallest unit is a penta- or hexanucleotide have been isolated.

Structure of Nucleic Acids. The assumption of a tetranucleotide structure for nucleic acid is based on the isolation of equimolecular proportions of the four bases.³³¹ In accord with this assumption are the results of potentiometric titration³³² and the results of changes in the optical rotation of solutions of nucleic acids as a function of hydron concentration.³³³ Further evidence was secured by the isolation of four nucleosides³³⁴ and four nucleotides on hydrolysis of nucleic acid.³³⁵

³²⁰ Altmann, *Arch. Anat. Physiol., Physiol. Abt.*, 524 (1889).

²³¹ Steudel, *Z. physiol. Chem.*, **49**, 406 (1906); Levene and Mandel, *Biochem. Z.*, **10**, 215 (1908).

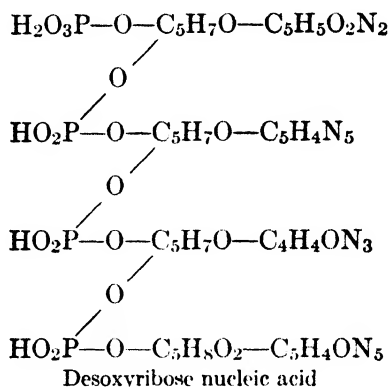
¹²² Levene and Simms, *J. Biol. Chem.*, **70**, 327 (1926).

³³³ Levene and Bass, *ibid.*, **74**, 727 (1927); Levene, Simms, and Bass, *ibid.*, **70**, 243, (1926).

³⁴ Levene and Jacobs, *Ber.*, **43**, 3154 (1910); Levene and La Forge, *Ber.*, **45**, 608 (1912).

³³⁸ Jones and Kennedy, *J. Pharmacol.*, **18**, 253 (1918); Levene, *Proc. Soc. Exptl. Biol. Med.*, **15**, 21 (1917); *J. Biol. Chem.*, **40**, 415 (1919).

The mode of union of the nucleotide to form the tetranucleotide unit has been a matter of much speculation. Most reliable information has been obtained in regard to the desoxyribosenucleic acids, thanks to the isolation of the diphosphoric esters of the pyrimidine nucleosides. Desoxyribosenucleosides possess only two hydroxyl groups. Hence the position of the phosphoric acid residues on the desoxyribose residue is determined by the position of the two hydroxyl groups. The formula below give a most satisfactory account of the facts known about desoxyribosenucleic acid.



An alternating arrangement of the purine and pyrimidine nucleotides is assigned to the substance in order to give a structural reason for the formation, on hydrolysis, of the diphosphopyrimidine nucleosides. The arrangement of the bases is in other respects arbitrary.

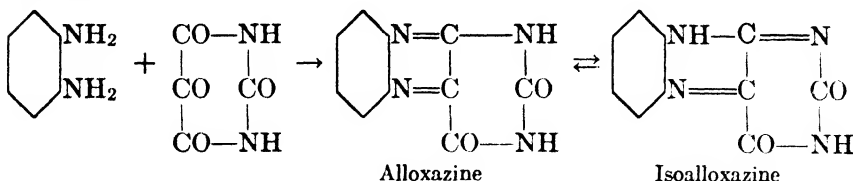
The arrangement of nucleotides in the ribosenucleic acids was formulated by analogy with that of desoxyribosenucleic acid. This view was confirmed by potentiometric titration, which made it possible to account for four primary and one secondary phosphoric acid group and was further confirmed by the nitrogen distribution, the theory requiring 20 per cent of nitrogen in form of the amino group. The theoretical proportion was obtained on analysis of the ribonucleic acids.

VITAMINS CONTAINING THE PYRIMIDINE RING*

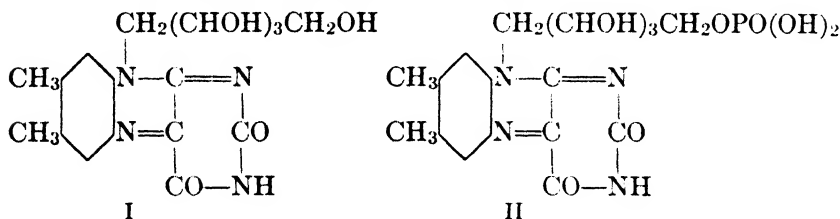
The rapid and steady progress made in recent years in the study of the chemistry of vitamins has led to the final conclusion that the pyrimi-

* References concerning vitamins: Karrer, *Helv. Chim. Acta*, **19**, 41 (1936); Wagner-Jauregg, *Angew. Chem.*, 318, 547 (1934); Kuhn, "Ann. Rev. Biochem.," Vol. 4, 490 (1935); *Bull. soc. chim. biol.*, **17**, 905 (1935); *Chemistry & Industry*, **52**, 981 (1933); *Angew. Chem.*, **48**, 29 (1935); **49**, 6 (1936); Kuhn, Rudy, and Wagner-Jauregg, *Ber.*, **66**, 1950 (1933); Kuhn, Wagner-Jauregg, Van Klaveren, and Vetter, *Z. physiol. Chem.*, **234**, 196 (1935); Harris, "Ann. Rev. Biochem.," Vol. 4, 332 (1935); Grewe, *Z. physiol. Chem.*, **242**, 89

dine ring occurs in both the growth-promoting vitamin B₂ (lactoflavin), and in the antineuritic vitamin B₁.³³⁶ Vitamin B₂ is a derivative of isalloxazine.



The accepted structure of vitamin B₂ is expressed by Formula I, namely—6,7-dimethyl-9-*d*-ribitylisoalloxazine.



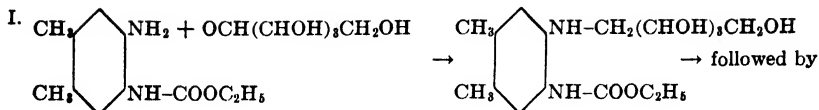
The carbohydrate functioning in the lactoflavin (I) is *d*-ribose,³³⁷ and it has been shown conclusively that this ribose derivative (I) is the growth-promoting substance present in vitamin B₂, but is not necessarily the antipellagra factor.³³⁸ The phosphoric acid combination expressed by Formula II is present in Warburg's yellow respiration ferment. This naturally occurring phosphate was prepared by treating lactoflavin (I) with phosphorus oxychloride in the presence of pyridine. Methods of synthesizing vitamin B₂ or lactoflavin have been applied successfully as follows:

(1936); Williams and Cline, *J. Am. Chem. Soc.*, **58**, 1505 (1936); Todd, Bergel, and Karimullah, *Ber.*, **69**, 217 (1936); Barger, Bergel, and Todd, *Ber.*, **68**, 2257 (1935); Windaus, Tschesche, and Grewe, *Z. physiol. Chem.*, **237**, 98 (1935); Kuhn and Vetter, *Ber.*, **68**, 2375 (1935); Ohdake, *Proc. Imp. Acad. (Tokyo)*, **10**, 95 (1934); Kimberly, O'Brien, and Peters, *Biochem. J.*, **29**, 701 (1935); Windaus, Tschesche, and Ruhkopf, *Nachr. Ges. Wiss. Göttingen Math.-physik. Klasse*, **342** (1932); Windaus, Tschesche, Ruhkopf, Laquer, and Schultz, *Z. physiol. Chem.*, **204**, 123 (1932). See, also, Sherman and Smith, "The Vitamins," American Chemical Society Monograph, No. 6, Chemical Catalog Co., New York City (1931); Harrow and Sherwin, "Chemistry of the Hormones," Williams and Wilkins Co., Baltimore (1934); Salmonsens, "Bibliographical Survey of the Vitamins, 1650-1930," Wodlinger, Chicago (1932).

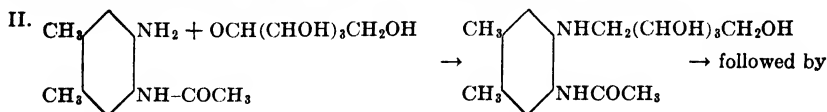
³³⁶ Nomenclature on "Aneurin:" Jansen, *Nature*, **136**, 259 (1935).

³³⁷ Kuhn, Rudy, and Weygand, *Ber.*, **68**, 625 (1935); v. Euler, Karrer, Malmberg, Schöpp, Benz, Becker, and Frei, *Helv. Chim. Acta*, **18**, 522 (1935); Karrer, v. Euler, Malmberg, Schöpp, and Benz, *Svensk Kem. Tid.*, **47**, 99 (1935).

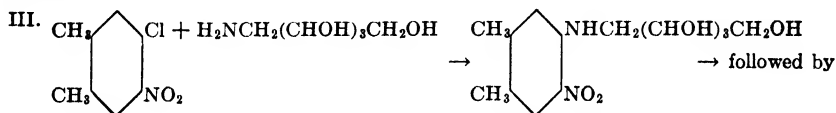
³³⁸ Elvehjem and Koehn, *J. Biol. Chem.*, **103**, 709 (1935); Karrer, Salomon, Schöpp, Benz, and Becker, *Helv. Chim. Acta*, **18**, 908 (1935).



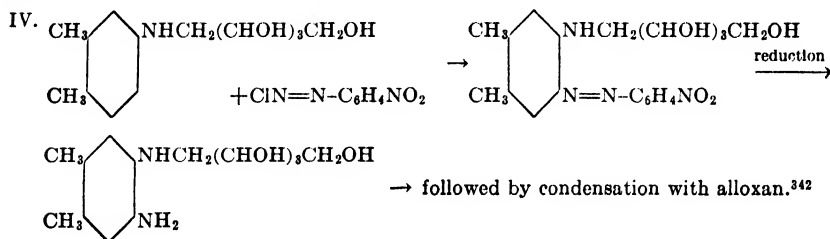
condensation with alloxan to give vitamin B₂ (C₁₇H₂₀O₆N₄) melting at 282°. ³³⁹



condensation with alloxan ³⁴⁰ to give vitamin B₂.



reduction to the amino compound and then condensation with alloxan to form the lactoflavin. ³⁴¹



Kuhn and his co-workers³⁴³ emphasize certain phases of their synthetic technique, laying stress on the use of palladium instead of nickel in the reductive condensations with *d*-ribose, and the use of boric acid in the condensations with alloxan. Condensations are also carried out successfully in glacial acetic acid solutions.

When lactoflavin (I) is irradiated in methyl alcohol solution, lumichrome (III) is formed.³⁴⁴ Irradiation in alkaline solution leads to the formation of lumilactoflavin (IV).³⁴⁵ Both of these irradiation products have been prepared synthetically.³⁴⁶

It is now generally accepted that the hydrochloride of the antineuritic

³³⁹ Karrer and co-workers, *Helv. Chim. Acta*, **18**, 69, 522 (1935).

³⁴⁰ Karrer and co-workers, *Ber.*, **68**, 216 (1935).

³⁴¹ Kuhn and Weygand, *Ber.*, **67**, 1939 (1934).

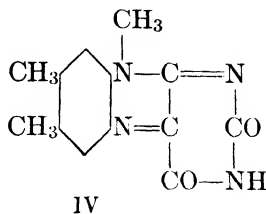
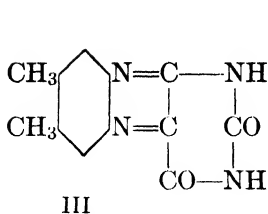
³⁴² Karrer and Meerwein, *Helv. Chim. Acta*, **18**, 1130 (1935).

³⁴³ Kuhn, Reinemund, Weygand, and Ströbele, *Ber.*, **68**, 1765 (1935).

³⁴⁴ Karrer, Salomon, Schöpp, Schlittler, and Fritzsche, *Helv. Chim. Acta*, **17**, 1010, 1165 (1934).

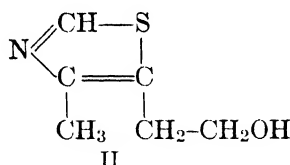
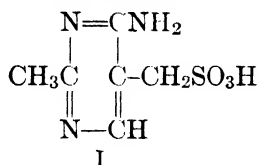
³⁴⁵ Kuhn and Rudy, *Ber.*, **68**, 300 (1935).

³⁴⁶ Kuhn, Reinemund, and Weygand, *Ber.*, **67**, 1460 (1934); Karrer, Salomon, Schöpp, Schlittler, and Fritzsche, *Helv. Chim. Acta*, **17**, 1010, 1165 (1934).



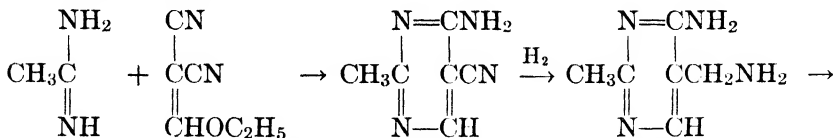
vitamin B₁ has the empirical formula C₁₂H₁₈ON₄Cl₂S. The first real progress in the elucidation of the structure of this vitamin was made by the American investigator R. R. Williams and his co-workers. They made the interesting observation that the hydrochloride of this vitamin is attacked by sodium sulfite in aqueous sulfur dioxide solution, suffering a molecular fission giving two characteristic products, both of which contain sulfur.³⁴⁷ One product was a base which yielded by oxidation with nitric acid an acid, C₅H₅O₂NS, that was identical with a substance previously obtained by Windaus and co-workers by oxidation of the vitamin B₁.³⁴⁸ This has been identified as a thiazole compound, 4-methyl-5-thiazolcarboxylic acid,³⁴⁹ and its precursor has been identified and proved by synthesis to be 4-methyl-5-β-hydroxyethylthiazole (II).³⁵⁰

The second product of cleavage of the vitamin by the action of sodium sulfite contains a pyrimidine ring, and is to be assigned the constitution expressed in Formula I.



Two syntheses of vitamin B₁ have already been applied successfully. They are as follows:

I. Grewe's method.³⁵¹



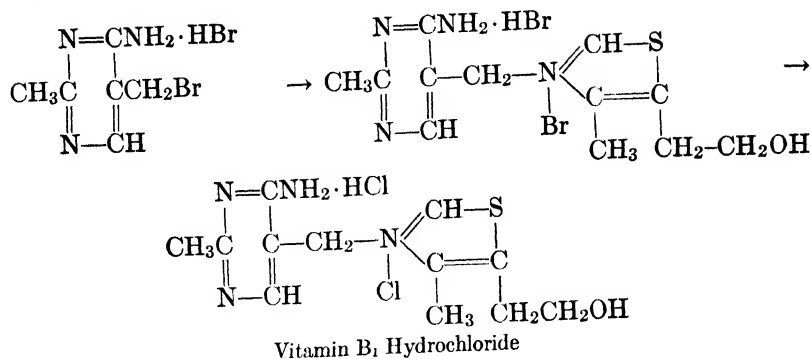
³⁴⁷ Williams, *J. Am. Chem. Soc.*, **57**, 229 (1935); Williams, Waterman, Keresztesy, and Buchman, *ibid.*, **57**, 536 (1935).

³⁴⁸ Windaus, Tschesche, and Grewe, *Z. physiol. Chem.*, **228**, 27 (1934); Buchman, Williams, and Keresztesy, *J. Am. Chem. Soc.*, **57**, 1849 (1935).

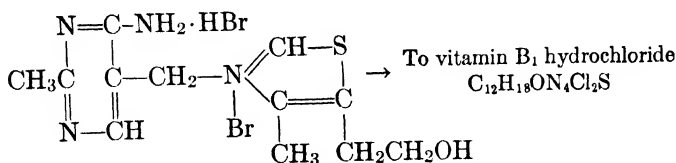
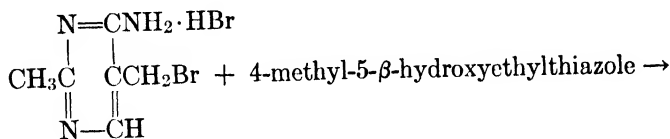
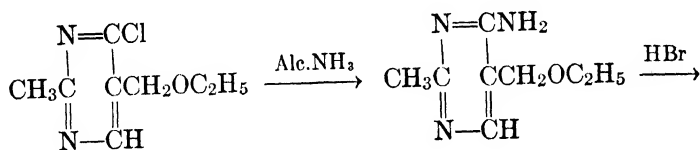
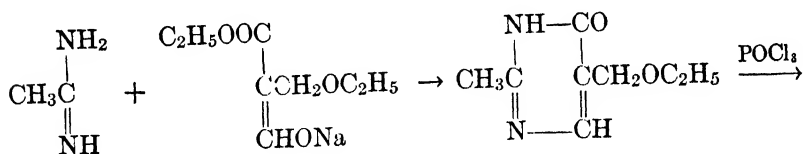
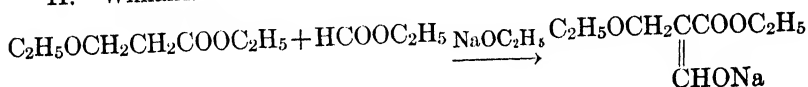
³⁴⁹ (a) Clarke and Gurin, *ibid.*, **57**, 1876 (1935); (b) Tomlinson, *J. Chem. Soc.*, 1030 (1935).

³⁵⁰ Clarke and Gurin, *J. Am. Chem. Soc.*, **57**, 1876 (1935).

³⁵¹ Grewe, *Z. physiol. Chem.*, **242**, 89 (1936). Synthesis accomplished only in part.



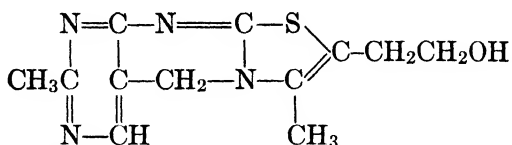
II. Williams and Cline's method.³⁵²



The synthetic vitamin B₁ hydrochloride was found identical with the natural vitamin in composition, ultra-violet absorption, and antineuritic potency.

³⁵² Williams and Cline, *J. Am. Chem. Soc.*, **58**, 1505 (1936); See also *ibid.*, **58**, 1063 (1936).

Oxidation of vitamin B₁ leads to the formation of thiochrome found in yeast.³⁵³ Oxidation of vitamin B₁ is accomplished successfully by action of potassium ferricyanide in alkaline solution³⁵⁴ and also by treatment with porphyrin.³⁵⁵



Dehydrovitamin B₁ or thiochrome

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³⁵³ Kuhn, Wagner-Jauregg, Van Klaveren, and Vetter, *Z. physiol. Chem.*, **234**, 196 (1935). See also Windaus, Tschesche, and Grewe, *ibid.*, **237**, 98 (1935).

³⁵⁴ Barger, Bergel, and Todd, *Nature*, **136**, 259 (1935); *Ber.*, **68**, 2257 (1935).

³⁵⁵ Kuhn and Vetter, *Ber.*, **68**, 2375 (1935).

CHAPTER 12

ALKALOIDS

LYNDON SMALL

University of Virginia

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INTRODUCTION

The term alkaloid, meaning alkali-like, is applied to naturally occurring basic nitrogen compounds, and is, in general usage, limited to those of plant origin. Most of the alkaloids have the nitrogen atom linked in a cyclic structure, are optically active, and show marked physiological activity, although a few substances classified as alkaloids are exceptional in respect to one or more of these characteristics. A variety of open-chain simple bases, as the cholines, amino acids, and phenylalkylamines, are distinguished from the true alkaloids by some authorities, under the name vegetable bases. The distinction is somewhat arbitrary, and ephedrine, mescaline, and a few similar bases will be here treated as alkaloids.

The nomenclature of the individual alkaloids has not been systematized, for historical reasons and because of the complexity of the structures involved. A great many important alkaloids have received names

derived from those of plants, as papaverine, hydrastine, berberine. A few are named from their physiological action, as morphine, narcotine, and emetine; several from their physical characteristics, as hygrine and porphyroxine; only one, pelletierine, has been named for an alkaloid chemist. The name of the principal alkaloid with a prefix or suffix is often applied to the minor alkaloids found in the same plant, as for example in the cinchona series; related bases are sometimes named by transpositions, as narcotine, cotarnine, and tarconine. It is customary to designate isomeric new bases (often transformation products of the natural alkaloid) with such prefixes as *iso*, *pseudo*, *allopseudo*, *neo*, *epi*, or with Greek letters (see the codeine isomers and the methylmorphimethines, p. 1079), or occasionally with a suffixed Roman letter.

The alkaloids as a class are well-crystallized colorless compounds; a few, notably arecoline, sparteine, the hygrines, and most members of the conine and nicotine groups, are liquids. The liquid alkaloids are generally oxygen-free. Colored alkaloids are rare; berberine is yellow, and the salts of sanguinarine are copper-red. Nearly all alkaloids form crystalline salts, which are often utilized in isolating or purifying the base; the acids usually employed are sulfuric, oxalic, perchloric, tartaric, salicylic, and the halogen acids. Most alkaloids react with alkyl halides, especially methyl iodide, to give crystalline addition products. Secondary amines give with methyl iodide the N-methylated hydriodide, tertiary amines give methiodides, which are of importance for degradative reactions. The so-called alkaloid reagents are used for the detection and often for the identification of minute amounts of the natural bases or their derivatives, and can be divided roughly into precipitants and color reagents. The precipitating reagents combine with alkaloids to give almost insoluble addition products, and thus may serve to demonstrate the presence of alkaloidal material, even in very small quantities, in drugs or plant extracts. A few of the reagents, however, are known to form precipitates with non-alkaloidal classes (proteins and glucosides). The alkaloid chemist utilizes the precipitants as convenient reagents for the approximate estimation of the amount of alkaloid remaining in aqueous solution after filtration or extraction. The precipitates often have a definite, constant composition, and can be employed for analysis; they sometimes crystallize in characteristic forms on the microscope slide, and permit preliminary identification of the alkaloid. Among the more important precipitating reagents may be mentioned Mayer's (potassium mercuric iodide), Sonnenschein's (phosphomolybdic acid), Knorr's (picrolonic acid), Hager's (picric acid), Wagner's (potassium triiodide), Dragendorff's (potassium bismuth iodide), Scheibler's (phosphotungstic acid), and Bertrand's (silicotungstic acid); further,

chloroplatinic and chloraureic acids, which are adapted to analytical use. In individual cases the precipitating reagents, especially picric acid, have been used to separate mixtures of alkaloids.

The color reagents mostly consist of dehydrating or oxidizing agents, or combinations of these, to which aldehydes may also be added. The alkaloidal residue obtained by evaporation of solutions in a porcelain dish is moistened with the reagent, and often warmed, and the color produced is compared with that from known samples. In certain cases, as for example, Lautenschläger's diazosulfanilic acid reagent for morphine, dilute solutions are employed, and the amount of alkaloid present can be determined with the colorimeter. The common color reagents are concentrated sulfuric acid solutions of such substances as formaldehyde (Marquis' reagent), nitric acid (Erdmann's), potassium dichromate (Luchini's), potassium permanganate (Wenzell's), or molybdic acid (Fröhde's).

Alkaloids are found almost exclusively in phanerogams, the seed-bearing plants, for the most part in dicotyledons, seldom in monocotyledons; occurrence in cryptogams is rare (ergot). The same species or genus may contain many different alkaloids, which are, however, usually related in structure. From the opium poppy, for example, ten members of the benzyloquinoline group have been isolated, which differ chiefly in the nature of peripheral groups, or in the degree of hydrogenation of the nucleus. The four morphine-type alkaloids found in the same plant differ from each other in the same way, and in theory, at least, can be related to the benzyloquinoline group by the establishment of a single new carbon-to-carbon linkage.^{1,2,3} It is indeed difficult to find any case where unrelated alkaloids occur in a single species. A given alkaloid is seldom present in different plant families; berberine, protopine, and the xanthine derivatives are exceptional in this respect. The alkaloidal content may be greatly influenced by selection and cultivation; planters have been especially successful in increasing the quinine yield from the cinchona tree in Java. The function of alkaloids in the plant is still a subject of speculation. The alkaloids are generally concentrated in the living tissue at points of intense cell activity, whence they are often cast aside and stored in such dead structures as the seed hulls or outer bark. They are regarded as by-products of plant metabolism (Tschirch), in contrast to the simple bases and betaines that probably constitute the building units for the formation of plant proteins. Other theories that have been advanced conceive alkaloids to

¹ Gulland and Robinson, *Mem. Proc. Manchester Lit. Phil. Soc.*, **69**, 79 (1925).

² Schöpf, *Ann.*, **452**, 211 (1927).

³ Awe, *Arch. Pharm.*, **272**, 466 (1934).

be reserve materials stored for protein synthesis; protective substances discouraging animal or insect attacks; plant stimulants or regulators similar to hormones; or detoxication products, rendered harmless to the plant by methylation, condensation, ring closure, and other synthetic processes.

Alkaloids occur usually in the form of salts of the common natural organic or inorganic acids, or of acids peculiar to the plant family, as meconic acid in the poppy, or quinic acid in cinchona. Occasionally the alkaloids are present in the free state, because of extremely weak basic properties, e.g., narceine and narcotine. More rarely they exist in combination with sugars, for example the glucoalkaloid solanine, or in the form of amides (piperine), or esters (atropine, cocaine) of organic acids. The crude alkaloid is separated from the powdered plant parts by extraction with water, alcohol, or dilute acids (hydrochloric, sulfuric, or acetic); or the vegetable material may be treated with alkali and the alkaloid extracted by organic solvents. For volatile alkaloids (nicotine and coniine groups), steam distillation is employed: The crude mixture of alkaloids obtained by these methods always contains coloring matter and resins, and is generally purified by repeated crystallization of sparingly soluble salts. Adsorbing agents (charcoals) are frequently used to remove color; occasionally the colored impurities can be destroyed by oxidation, as is the practice in cocaine manufacture.⁴ The individual alkaloids are usually separated through differences in solubility of their various salts. The separation may sometimes be accomplished by utilizing differences in basicity of the alkaloids, i.e., fractional extraction or precipitation. In this method, which was developed by Gadamer, a solution of the mixed alkaloids in dilute acid is treated with successive small portions of ammonia or sodium hydroxide, and the liberated alkaloid is extracted with an organic solvent after each addition of alkali. The first fractions will contain the weakly basic alkaloids, the last fractions the more strongly basic. Conversely, a solution of the mixed alkaloids in benzene, ether, or chloroform may be extracted with many small portions of dilute acid, the strongest bases being extracted first.

The first step in structure determination consists in isolating the nitrogen-containing portion of the alkaloid, whether by simple liberation from salts, or by hydrolytic processes as exemplified in the case of the glucoalkaloids and cocaine types. Hydrolysis of new alkaloids must, however, be employed with caution, since in some types of alkaloid structure, for example narcotine, hydrastine, thebaine, strychnine, the basic portion itself may be split or undergo racemization. After deter-

⁴ Schwyzer, "Die Fabrikation der Alkaloide," Springer, Berlin (1927); "Die Fabrikation pharmazeutischer und chemisch-technischer Produkte," Springer, Berlin (1931).

mining the empirical formula and the optical rotatory power of the pure alkaloid, the chemist proceeds to ascertain the function of oxygen and nitrogen, how the molecule may be broken into simple fragments, and what the fundamental ring system may be. The presence of oxygen as a phenolic hydroxyl is shown by alkali-solubility, ferric chloride reaction, acylation, and alkylation; in the form of an alcoholic hydroxyl by reaction with phosphorus chlorides or thionyl chloride, by acetylation, or occasionally by dehydration or oxidation. Carboxyl groups (arecaine, narceine), confer solubility in sodium carbonate or ammonia, and their presence may be demonstrated by esterification. Ether-linked methoxyl groups and acetal-linked dioxymethylene groups occur frequently. Methoxyl groups can be estimated quantitatively by the method of Zeisel^{5,6} or of Vieböck,⁷ which involves boiling the substance with concentrated hydriodic acid and determining the amount of methyl iodide formed. The detection and quantitative estimation of the methylenedioxy group are accomplished by reactions in which formaldehyde is split out by means of sulfuric acid.⁶ No other alkoxyl groups have ever been found, a fact that indicates the importance of formaldehyde in the phytochemical synthesis of alkaloids. Many alkaloid structures are so stable that methoxyl or methylenedioxy groups may be split without other structural changes, whereby the corresponding hydroxy bases are obtained. For this purpose, constant-boiling hydrobromic acid, or aluminum bromide, have proved particularly useful.^{8,9} Carbonyl groups (cryptopine, narceine) may be identified by the usual methods; lactone (narcotine, hydrastine), lactam, or betaine (arecoline, hypaphorine) groups are usually detected by hydrolysis.

The determination of methyl groups on nitrogen is carried out by the method of Herzig and Meyer,^{6,10} which consists in heating the alkaloid hydriodide at 200–300° and estimating the methyl iodide formed. This process may be carried out in combination with the Zeisel analysis for methoxyl groups. Occasionally the methyl group on nitrogen (higher N-alkyl groups are never found) can be replaced by hydrogen through the action of cyanogen bromide, nitrous acid, alkaline permanganate, or other reagents, yielding secondary amines. These are distinguished by *nor* prefixed to the alkaloid name, but the same prefix is sometimes used to designate bases obtained by demethylation at oxygen.

⁵ Zeisel, *Monatsh.*, **6**, 989 (1885); **7**, 406 (1886).

⁶ Meyer, "Analyse und Konstitutionsermittlung organischer Verbindungen," Springer, Berlin (1922).

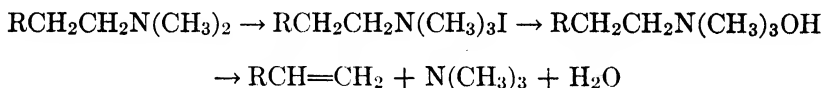
⁷ Vieböck, *Ber.*, **63**, 2818, 3207 (1930).

⁸ Schöpf and Thierfelder, *Ann.*, **497**, 22 (1932).

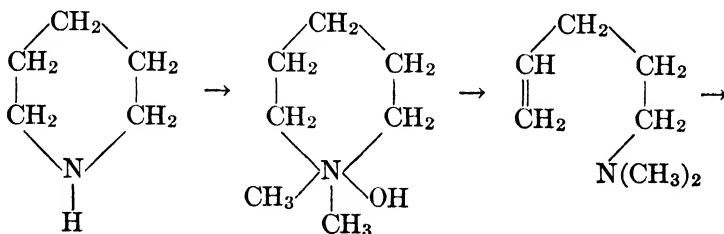
⁹ Mosettig and Burger, *J. Am. Chem. Soc.*, **52**, 2988 (1930).

¹⁰ Herzig and Meyer, *Monatsh.*, **15**, 613 (1894); **16**, 599 (1895); **18**, 379 (1897).

With few exceptions the nitrogen in alkaloids is in a ring structure, and can be only secondary or tertiary. It is often difficult to distinguish between these two forms. Tests for the secondary amino group which depend upon reactions of active hydrogen cannot be evaluated until the number of other active hydrogens in the molecule is known. Nitrogen is generally assumed to be tertiary if the usual reactions^{6,11,12} for secondary nitrogen are negative.* The ability to form amine oxides with 30 per cent hydrogen peroxide, and to react with 1,5-dibromopentane¹³ with formation of pentane diammonium bromides, is sometimes used to characterize tertiary bases. The most generally applicable method for ascertaining structure is exhaustive methylation, also known as the Hofmann degradation (p. 713). This depends upon the tendency of many quaternary ammonium hydroxides to decompose with loss of water and scission of a carbon-to-nitrogen linkage when heated,† and often gives immediate structural information. With an open-chain tertiary amine, a single methylation and decomposition suffices to eliminate nitrogen as trimethylamine.



If, on the other hand, two of the nitrogen valences are involved in a hydrogenated ring structure, the first decomposition yields an unsaturated open-chain amine, with which the process must be repeated before nitrogen can be split out and the carbon skeleton exposed.



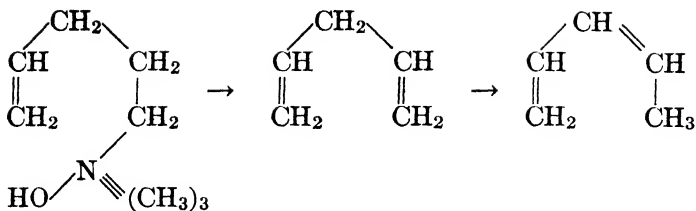
¹¹ Houben, "Die Methoden der organ. Chemie," 2nd. ed., Thieme, Leipzig (1925), Vol. IV, pp. 369, 502.

¹² Rosenthaler, "Der Nachweis organ. Verbindungen," 2nd ed., Enke, Stuttgart (1923), p. 514.

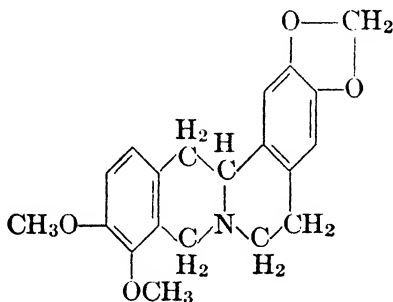
* Such reactions must be interpreted with caution. Some tertiary bases (e.g., apomorphine, morphothebaine) yield N-benzoyl derivatives through ring scission.

¹³ von Braun, *Ber.*, **41**, 2156 (1908).

† For a discussion of the probable mechanism see Schlenk-Bergmann "Ausführliches Lehrbuch der organischen Chemie," Deuticke, Leipzig and Vienna (1932), Vol. I, p. 55.



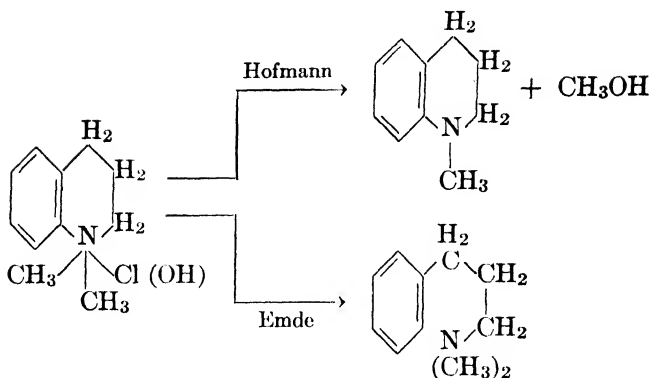
See for example the degradation of pseudopelletierine to cyclooctadiene, p. 1035. In accordance with a suggestion of Willstätter,¹⁴ the unsaturated amine formed in the first step of exhaustive methylation usually receives the prefix *des*-. The suffix -methine is also sometimes used to designate these compounds (see the codeine series). Where nitrogen is linked in ring structures through three valences, three methylations and decompositions are necessary to eliminate the nitrogen. This is true of canadine or tetrahydroberberine,¹⁵ in which the following ring system is present:



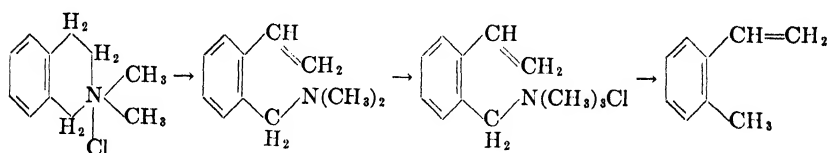
The Hofmann degradation is not applicable to all types of alkaloids; it fails with unhydrogenated pyridine, quinoline, and isoquinoline derivatives, and with hydrogenated quinolines. A useful modification, the Emde degradation, involves treating an alcoholic or aqueous solution of the quaternary halide with sodium amalgam. The Emde degradation may yield the same degradation product as the Hofmann method, or a reduced derivative, sometimes a mixture of the two. It often succeeds with ring systems that cannot be degraded according to Hofmann. Tetrahydrodimethylquinolinium halides, for example, split off methyl alcohol to give *N*-methyltetrahydroquinoline when heated with alkali. By Emde's process, the principal product is γ -dimethylaminopropylbenzene.

¹⁴ Willstätter, *Ann.*, **317**, 268 (1901).

¹⁵ McDavid, Perkin, and Robinson, *J. Chem. Soc.*, **101**, 1218 (1912).

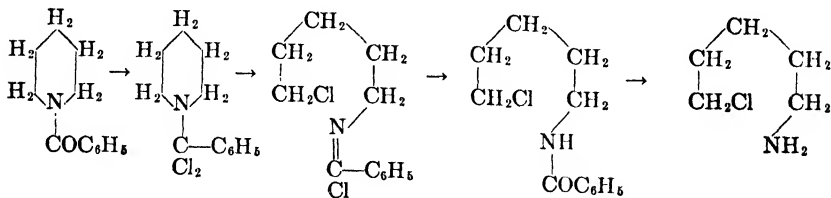


With tetrahydrodimethyloisoquinolinium halides, both methods yield the same primary product, (*o*-vinylbenzyl)-dimethylamine, but further degradation by Hofmann is unsuccessful, whereas application of the Emde method results in *o*-methylstyrene.^{16, 17}



It has been discovered recently¹⁸ that some quaternary ammonium halides can be degraded smoothly by catalytic hydrogenation, but the method has not yet found application in the alkaloid series.

Two other methods for opening nitrogen-containing rings (pp. 714, 716) were devised by J. von Braun.¹⁹ One of these, applicable to cyclic secondary amines, consists in treating the benzoyl derivative of the amine with phosphorus halides. From benzoylpiperidine for example, ϵ -chloro- α -methylamine is obtained.



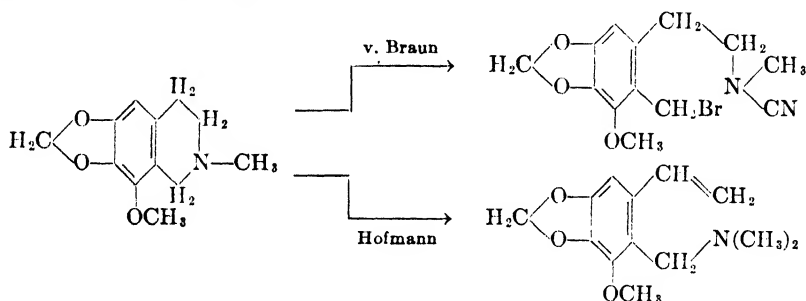
¹⁶ Emde and Kull, *Arch. Pharm.*, **272**, 469 (1934).

¹⁷ Emde, *Ann.*, **391**, 88 (1912).

¹⁸ Emde and Kull, *Arch. Pharm.*, **274**, 173 (1936).

¹⁹ von Braun, *Ber.*, **37**, 2915, 4723 (1904); **40**, 3914 (1907).

When the reaction is carried out at a higher temperature, 1,5-dichloropentane and benzonitrile are the products. The second von Braun degradation makes use of cyanogen bromide, CNBr. This reagent reacts with tertiary nitrogen compounds to break one carbon-to-nitrogen linkage, the cyano group becoming attached to nitrogen and the bromine atom to carbon. Cyclic N-alkyl compounds may be dealkylated with formation of a cyclic N-cyano derivative, or the ring may be opened with formation of a cyanamino derivative, depending upon the structural features adjacent to the nitrogen atom. The cyanogen bromide degradation is of interest because it often succeeds with compounds that resist the Hofmann degradation, further, because it opens the ring at a different point from the latter. The degradation of hydrocotarnine (p. 1075), for example, proceeds thus:²⁰



Other vigorous degradations are often employed to determine the fundamental structures present. Oxidative methods have been widely used; see, for example, nicotine, cinchonine, papaverine. Oxidizing agents employed are chosen according to the degree of degradation desired and the stability of the structures present. Mild oxidizing agents, as silver acetate, mercurous acetate, or alkaline potassium ferricyanide, may cause only partial dehydrogenation. Chromic acid and alkaline permanganate have been used most frequently, and by control of temperature and concentration it is often possible to oxidize in steps until only the most resistant nuclei remain unattacked. Lead peroxide or manganese dioxide in sulfuric acid, nitric acid, hydrogen peroxide, and alkaline solutions of bromine or iodine have been used in individual cases. Distillation over hot zinc dust breaks the molecule down to stable ring systems; morphine gives phenanthrene, cinchonine yields quinoline and picoline, strychnine gives lutidine and carbazole. Other reductive methods, especially sodium and alcohol, and catalytic hydrogenation, have helped to establish relationships between alkaloids that differ in

²⁰ von Braun, *Ber.*, **49**, 2624 (1916).

degree of oxidation, or owe their isomerism to differences in the position of unsaturated linkages. Fusion with alkali, heating with bromine or phosphorus halides, and similar drastic reactions are often used in degrading the alkaloids to known substances. From the fragments thus obtained it is sometimes possible to make a reasonable structural picture of the alkaloid itself.

The constitution of a considerable number of alkaloids is now known with certainty, and for many the structural formula has been confirmed by synthesis. Especially noteworthy are the methods developed in recent years by C. Schöpf and G. Hahn for the synthesis of alkaloids under conditions comparable to those existing in the living plant. These investigations, which bring welcome support to the inspiring speculations of Winterstein and Trier, and Robert Robinson, are summarized in the concluding paragraphs of this chapter. The discovery of new and often medicinally important alkaloids is proceeding more rapidly than structure elucidation, and there still remain whole groups of long-known valuable alkaloids concerning whose structure there is little knowledge (for example, the aconite and veratrine groups), as well as many individual alkaloids whose empirical formula is still uncertain. There are few fields of organic chemistry where so many unsolved problems lie at hand. It is possible to discuss here only a limited number of the more important alkaloids, which have been chosen as representatives of various heterocyclic systems most frequently found in nature. Caffeine, theobromine, and theophylline, because of their purine structure, are treated elsewhere (p. 974). For more complete information, the numerous exhaustive textbooks on alkaloids must be consulted.²¹

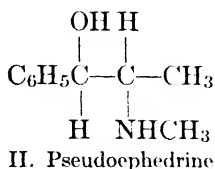
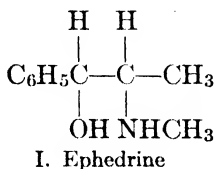
PHENYLALKYLAMINE GROUP

The phenylalkylamine bases ephedrine and hordenine depart from the conventional alkaloid definition in possessing an open-chain amine structure. Ephedrine has in other respects typical alkaloid properties, and especially because of its physiological action deserves discussion among the alkaloids. Hordenine, because of its obvious relationship to the cyclic bases from *Anhalonium* varieties, is treated under the mescal alkaloids (p. 1063).

Ephedra Bases. The Chinese herb known as Ma Huang, which has been used in the alleviation of a variety of ailments for some 5000 years, consists principally of the dried parts of *Ephedra sinica*

²¹ Henry, "The Plant Alkaloids," Churchill, London (1924); Schmidt-Grafe, "Alkaloide," Urban and Schwarzenberg, Berlin (1920); Seka, "Alkaloide," Urban and Schwarzenberg, Berlin (1927, 1933). Other textbooks and review articles are listed under "General References" on p. 1112.

or *E. equisetina*. At least six bases are present, of which the most important is *l*-ephedrine. It is accompanied by *d*-pseudoephedrine, *l*-methylephedrine, *d*-methylpseudoephedrine, *l*-norephedrine and *d*-norpseudoephedrine. All these constituents, as well as their optical antipodes and racemates, have been synthesized.



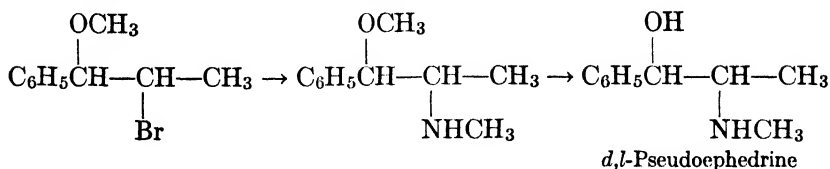
Ephedrine contains two dissimilar asymmetric carbon atoms, so that four optically active isomers (p. 164) are possible. Of these, the natural bases *l*-ephedrine and *d*-pseudoephedrine are diastereoisomers, and are mutually interconvertible. Their optical opposites are known only by synthesis. The first structural determination in the series was carried out by Ladenburg,²² who succeeded in demonstrating the nature of *d*-pseudoephedrine. It is a secondary base containing an alcoholic hydroxyl group, as indicated by the formation of a nitroso derivative and a dibenzoyl compound. The presence of a methyl group on nitrogen is evident from the appearance of methylamine when the base is degraded with hydrochloric acid, and the simultaneous formation of methylamine homologs shows that the methylamino group is not located at the end of a chain. Oxidation of pseudoephedrine gives benzoic acid or benzaldehyde, pointing to a hydroxyl on the carbon adjacent to the benzene ring. Evidence from these degradations, therefore, shows the probable structure for ephedrine and pseudoephedrine to be that of a propylbenzene carrying a hydroxyl and a methylamino group in the 1- and 2-positions of the side chain.

The formulas derived for the ephedra bases from degradative reactions have been confirmed by numerous syntheses.²³ Späth and Göhring prepared all the ephedrine isomers from 1-phenyl-1-methoxy-2-bromopropane. This was converted with methylamine to the corresponding 2-methylaminopropane, which, on treatment with hydrobromic acid, yielded 1-phenyl-1-hydroxy-2-methylaminopropane, racemic pseudoephedrine. The racemic base was resolved into the known (natural) *d*-pseudoephedrine and its enantiomorph *l*-pseudoephedrine, or isomer-

²² Ladenburg and Oelschlägel, *Ber.*, **22**, 1823 (1889).

²³ Eberhard, *Arch. Pharm.*, **253**, 62 (1915); **253**, 97 (1920); Späth and Göhring, *Monatsh.*, **41**, 319 (1920); (d) Nagai and Kanao, *Ann.*, **470**, 157 (1929); Manske and Johnson, *J. Am. Chem. Soc.*, **51**, 580 (1929); Skita and Keil, *Ber.*, **62**, 1142 (1929).

ized to *d,l*-ephedrine, which could likewise be resolved into the enantiomorphic *d*- and *l*- (natural) ephedrines.

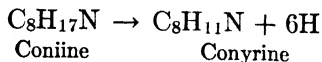


The action of acids converts *l*-ephedrine to *d*-pseudoephedrine, and prolonged heating with hydrochloric acid reverses this change. The rearrangement apparently takes place through replacement of the hydroxyl group by halogen, followed by hydrolysis of the 1-phenyl-1-halogeno-2-methylaminopropane with inversion at the number one carbon atom.²⁴ The configuration of *l*-ephedrine and of *d*-pseudoephedrine as in formulas I and II was established by Freudenberg through relationships to synthetic material of known configuration.²⁵ The synthesis of the natural ephedrine homologs is described^{23d} by Nagai and Kanao.

Ephedrine has a strong mydriatic action. It contracts the blood vessels and causes a prolonged rise in blood pressure. Its astringent action on mucous membrane is utilized in treating allergic conditions such as hay-fever and asthma, and in shrinking engorged nasal tissues.

PYRIDINE GROUP—HEMLOCK, PEPPER, POMEGRANATE, ARECA NUT, AND CASTOR-BEAN ALKALOIDS

Hemlock Alkaloids. The hemlock herb or spotted cowbane, *Conium maculatum*, contains five alkaloids, coniine $\text{C}_8\text{H}_{17}\text{N}$, γ -coniceine $\text{C}_8\text{H}_{15}\text{N}$, conhydrine $\text{C}_8\text{H}_{17}\text{ON}$, pseudoconhydrine $\text{C}_8\text{H}_{17}\text{ON}$, and N-methylconiine $\text{C}_9\text{H}_{19}\text{N}$, in combination with malic and caffeic acids. Coniine was isolated in 1831, but its constitution was not determined until about fifty years later (Hofmann).²⁶ Coniine is a strongly alkaline, dextrorotatory liquid, of penetrating odor and burning taste. When its hydrochloride is distilled with zinc dust, a new base, conyryne, containing six less hydrogen atoms, is formed. Conyryne can be reduced again to (optically inactive) coniine with concentrated hydriodic acid.

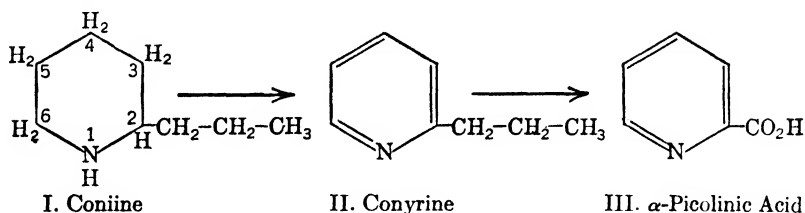


²⁴ Emde, *Helv. Chim. Acta*, **12**, 365 (1929); Emde and Spaenhauer, *ibid.*, **13**, 3 (1930).

²⁵ Freudenberg, Schoeffel, and Braun, *J. Am. Chem. Soc.*, **54**, 234 (1932); Freudenberg and Nikolai, *Ann.*, **510**, 223 (1934).

²⁶ Hofmann, *Ber.*, **18**, 5, 109 (1885).

Further information on the structure of coniine was obtained by the oxidation of conyryne, which yielded α -picolinic acid.



Since two carbon atoms were lost in the oxidation, conyryne was thus shown to be an α -propylpyridine. It was found not identical with the known α -isopropylpyridine and was therefore assigned the alternative formula, α -*n*-propylpyridine. Coniine is the dextro form of α -*n*-propylpiperidine.

Formula I is in accord with the behavior of coniine as a secondary amine in its reactions with acylating agents and with nitrous acid, and found confirmation in Ladenburg's synthesis²⁷ in 1886, the first synthesis of an alkaloid. In general, when pyridinium alkyl halides are heated under pressure, the alkyl group shifts to the α - or γ -position, yielding the corresponding alkylpyridines. Ladenburg's attempts to prepare conyryne by this method failed because of isomerization of the *n*-propyl group to isopropyl under the drastic conditions involved. The coniine synthesis was finally accomplished by condensation of α -picoline with paraldehyde and reduction of the condensation product to α -propylpiperidine with sodium and alcohol. The optically inactive product yielded on resolution with tartaric acid a dextrorotatory base identical with coniine (see also Hess).²⁸

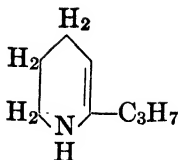
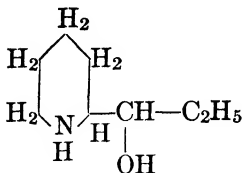
γ -Coniceine contains two hydrogen atoms less than coniine. It is optically inactive, hence the asymmetric carbon atom of coniine must be involved in the unsaturation; on reduction, γ -coniceine gives *d,l*-coniine. γ -Coniceine can be prepared from chloro- or bromo-coniine by the action of alkali, or from conhydrine by dehydration, facts which find an explanation in formula IV. γ -Coniceine was synthesized by Gabriel.²⁹

Conhydrine represents a coniine in which an alcoholic hydroxyl group replaces a hydrogen atom in the side chain. The position of this hydroxyl was long uncertain, but has been proved by the identity of the product of N-methylation and oxidation, methylconhydrinone (VI)

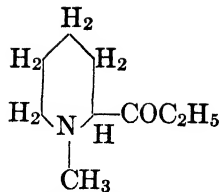
²⁷ Ladenburg, *Ber.*, **19**, 439, 2578 (1886).

²⁸ Hess and Weltzien, *Ber.*, **53**, 139 (1920).

²⁹ Gabriel, *Ber.*, **42**, 4059 (1909).

IV. γ -Coniceine

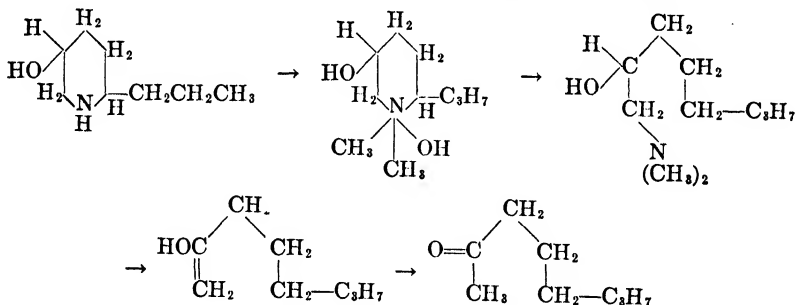
V. Conhydrine



VI. Methylconhydrinone

with the synthetic 1-(α -N-methylpiperidyl)-1-propanone.³⁰ Conhydrine can be reduced to coniine. On oxidation it yields either the ketone *d,l*-conhydrinone, or pipercolinic acid; the former is identical with synthetic 1-(α -piperidyl)-1-propanone.

Pseudoconhydrine is a structural isomer of conhydrine, which it resembles closely. It can be transformed through iodoconiine to *d*-coniine, or dehydrated to pseudoconiceine, an isomer of the γ -coniceine mentioned above. Späth³¹ showed by degradation that pseudoconhydrine has the hydroxyl group in the piperidine ring, at the 5-position. The pseudoconhydrinemethine resulting from the first step in the Hofmann degradation was hydrogenated; the dihydro derivative, on oxidation yielded enanthic acid (*n*-heptylic acid), or on further degradation gave 1,2-epoxyoctane. A portion of the dihydro methine, undergoing the normal Hofmann degradation, gave 2-octanone, leaving no doubt as to the position of the hydroxyl in pseudoconhydrine.



N-Methylconiine occurs naturally in both *d*- and *l*-forms. *d*-N-Methylconiine can be prepared from coniine by methylation, the *d,l*-form from the pomegranate alkaloid methylisopelletierine (p. 1036) by reduction.

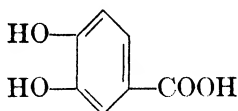
The conium alkaloids cause paralysis of the motor nerve endings, and all are poisonous. The hemlock drink used in ancient times to inflict the death penalty owed its toxicity to these bases.

³⁰ Hess and Grau, *Ann.*, **441**, 101 (1925); Späth and Adler, *Monatsh.*, **63**, 127 (1933).

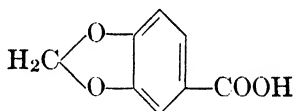
³¹ Späth, *Ber.*, **66**, 591 (1933).

Pepper Alkaloids. The alkaloid piperine, $C_{17}H_{19}O_3N$, occurs to the extent of about 5 to 9 per cent in the fruit of *Piper nigrum*, the source of black and of white pepper; it is present in lesser amounts in other *Piper* species. The sharp taste of pepper is apparently not due to piperine, but rather to an isomer, chavicine.³² A third base, piperovatine, is also known.

Piperine is a very weak, optically inactive base, yielding on hydrolysis piperic acid and piperidine. The latter substance first became known from this source. Piperic acid is unsaturated, and adds four atoms of bromine or of hydrogen. On oxidation with permanganate, it gives piperonal, and finally piperonylic acid. Piperonylic acid breaks down to protocatechuic acid and carbonaceous products when heated with hydrochloric acid at 170° ; conversely, it can be prepared from protocatechuic acid by the action of methylene iodide and alkali. Its constitution as the methylene ether of protocatechuic acid is evident.

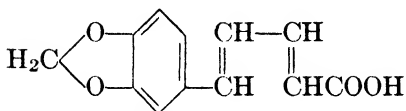


I. Protocatechuic acid

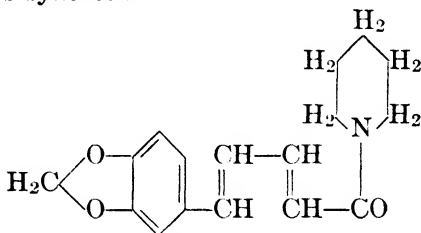


II. Piperonylic acid

Piperic acid differs from piperonylic acid by C_4H_4 , which must be located between the aromatic nucleus and the carboxyl group in order to explain the oxidation of piperic acid. The formula thus obtained (III) was confirmed by Ladenburg's synthesis.³³



III. Piperic acid



IV. Piperine

Piperine is the amide of piperic acid with piperidine, and was prepared by Rügheimer³⁴ from piperoyl chloride and piperidine before either of the components had been synthesized. Except as a local irritant, piperine is practically without physiological action.

Chavicine is the piperidine amide of chavicic acid. The latter

³² Ott and Lüdemann, *Ber.*, **57**, 214 (1924).

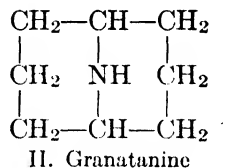
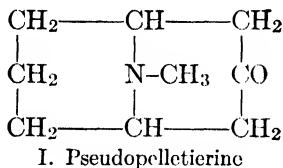
³³ Ladenburg and Scholtz, *Ber.*, **27**, 2958 (1894).

³⁴ Rügheimer, *Ber.*, **15**, 1390 (1882).

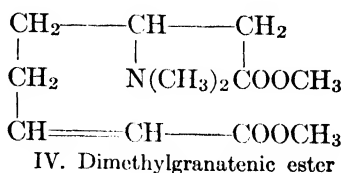
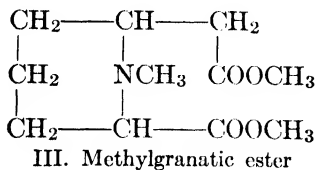
is a geometrical isomer (*cis-cis* form) of piperic acid (*trans-trans* form).³⁵

Pomegranate Alkaloids. The bark of the pomegranate tree (*Punica granatum*) contains four or more low-melting or liquid alkaloids discovered in 1877 by Tanret, and named pelletierines in honor of the French alkaloid chemist Pelletier. The existence and nomenclature of some of the bases have been the subjects of considerable dispute, and only those of well-established constitution will be discussed here.

The chief alkaloid, pseudopelletierine (N-methylgranatonine), $C_9H_{15}ON$, contains two piperidine rings having nitrogen and two carbon atoms in common, and is closely related to tropinone (p. 1048). The nitrogen atom is tertiary, and carries a methyl group. The single oxygen atom is linked in a ketone group, which stands between two methylene groups, as is shown by the ability of pseudopelletierine to form an oxime, and dibenzylidene or diisonitroso derivatives. By reduction at the carbonyl group, a secondary alcohol, methylgranatoline, is obtained. This base can be converted through a series of intermediates to granatanine, the parent substance of the series. Granatanine is a homolog of norhydrotropidine in the tropine series.³⁶



The constitution of pseudopelletierine rests upon degradations and synthesis. Distillation of granatanine hydrochloride over zinc dust gives α -propylpyridine, a degradation parallel to that of norhydrotropidine to α -ethylpyridine.³⁷ Pseudopelletierine yields on oxidation a dibasic acid, methylgranatic acid, which still has a piperidine ring intact and contains the same number of carbon atoms as the starting material. Exhaustive methylation of methylgranatic acid leads through IV and V

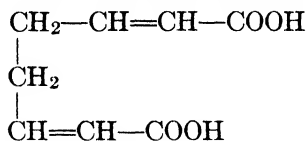


³⁵ Ott and Eichler, *Ber.*, **55**, 2653 (1922).

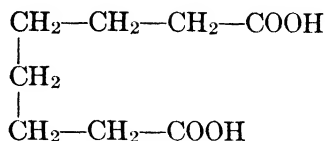
³⁶ Ciamician and Silber, *Ber.*, **26**, 2738 (1893).

³⁷ Ciamician and Silber, *Ber.*, **27**, 2850 (1894).

to suberic acid,³⁸ demonstrating the presence of an unbranched eight-carbon chain in pseudopelletierine.

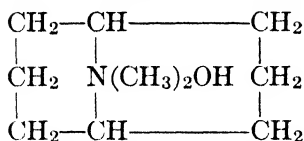


V. Homopiperylenedicarboxylic acid

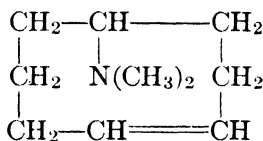


VI. Suberic acid

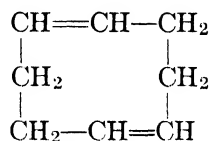
Pseudopelletierine itself was degraded by Willstätter³⁹ through methylgranatanine to cyclooctadiene, which could be reduced to cyclooctane, or dehydrogenated (by the device described on p. 1049 for the tropilidene synthesis) to the cyclooctatetraene so significant to theories concerning aromatic ring structure (p. 64).



VII. Methylgranatanine methohydroxide

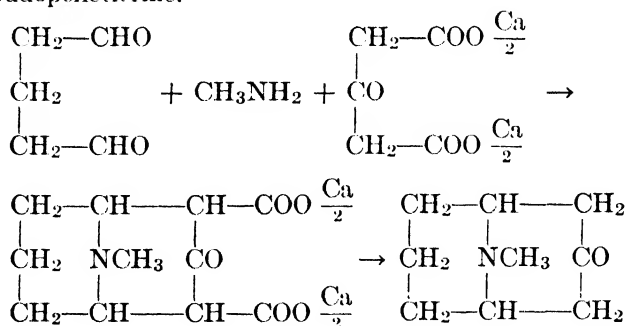


VIII. des-Dimethylgranatanine



IX. Cyclooctadiene

Pseudopelletierine was synthesized by Menzies and Robinson⁴⁰ through a reaction developed as a result of theoretical speculations on the mode of formation of the alkaloid in the plant. Glutaric aldehyde, methylamine, and calcium acetonedicarboxylate were condensed, the product acidified, and the free dibasic acid distilled in a high vacuum, yielding pseudopelletierine.



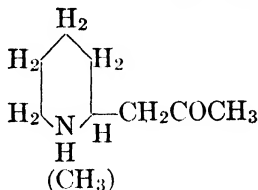
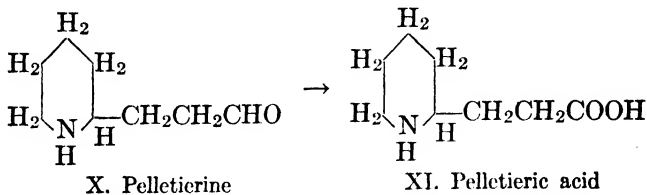
The synthesis of pseudopelletierine by a parallel reaction, under strictly physiological conditions, is discussed at the end of this chapter.

³⁸ Piccinini, *Gazz. chim. ital.*, [II] **29**, 104 (1899) [*Chem. Zentr.*, II, 808 (1899)].

³⁹ Willstätter and Waser, *Ber.*, **43**, 1176 (1910); **44**, 3423 (1911).

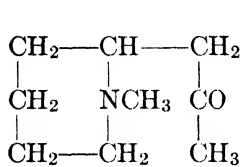
⁴⁰ Menzies and Robinson, *J. Chem. Soc.*, **125**, 2163 (1924).

Pelletierine (often called punicine), $C_8H_{15}ON$, is an aldehyde possessing the carbon-nitrogen skeleton of coniine, to which it can be reduced. The oxime of pelletierine gives on dehydration a nitrile, which is saponifiable to pelletieric acid, identical with α -piperidylpropionic acid, whence the structure X follows for pelletierine. This alkaloid, in spite of its simple formula, has not been synthesized.

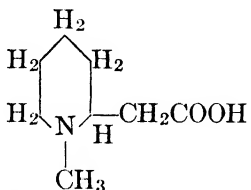
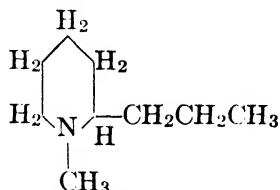


XII. Isopelletierine (Methylisopelletierine)

Isopelletierine and methylisopelletierine occur in very small amounts in pomegranate bark. The bases react readily with ketone reagents, and the course of oxidation shows that the carbonyl group must be in the side chain. Methylisopelletierine yields on oxidation *N*-methylpipercolinic acid, on reduction *d,l*-methyleconiine (p. 1032).



XIIa

XIII. *N*-Methylpipercolinic acid

XIV. Methyleconiine

The position of the carbonyl group in the side chain was determined by Hess^{30,41} and Meisenheimer⁴² through the identity of methylisopelletierine with 1-(α -*N*-methylpiperidyl)-2-propanone. A slight rearrangement of the methylisopelletierine formula (XIIa) shows the relationship of the piperidine type pomegranate alkaloids to the condensed ring system of pseudopelletierine.

Pelletierine, usually as a mixture of the pomegranate alkaloids con-

⁴¹ Hess and Littmann, *Ann.*, **494**, 7 (1932).

⁴² Meisenheimer and Mahler, *Ann.*, **462**, 301 (1928).

sisting chiefly of pseudopelletierine and isopelletierine, is used as an anthelmintic; it acts specifically on tapeworms.

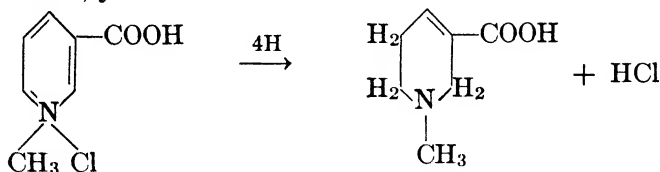
Areca Nut Alkaloids. The fruit of the betel palm, *Areca catechu*, is used as a mild stimulant and narcotic by some 200,000,000 persons in India, the Philippines, and the islands of the Pacific and Indian Oceans. Betel chewing is one of the most widespread habits of man. The chew usually consists of a piece of areca nut rolled in a leaf of the betel pepper (*Piper betle*) with some lime and a little gambir, tobacco, or catechu. The combination is chewed throughout the day, and often held in the mouth at night. It stimulates excessive salivation, and the saliva is colored blood-red by the action of the lime and gambir on the coloring matter of the areca nuts. The teeth are blackened rapidly. The addict experiences a feeling of well being, good humor, and contentment. The craving for the drug is intense, but the habit does not appear to cause any degeneration.⁴³ Of the five alkaloids that have been isolated from areca nuts, arecoline is the most important in respect to physiological action, but other substances present in the nuts probably contribute to the intoxicating effect.

Arecoline, $C_8H_{13}O_2N$, is an optically inactive liquid base, present to the extent of about 0.1 per cent in areca nuts. On hydrolysis it is split into methyl alcohol and arecaidine, $C_7H_{11}O_2N$, another alkaloid that is found in smaller amounts in the nuts. Arecaidine is amphoteric; it forms salts both with acids and with alkalis. On esterification with methyl alcohol it is converted to arecoline. The nitrogen atom carries a methyl group that can be split off as methyl chloride by hydrochloric acid at 240° ; on treatment with lime, methylamine is formed.

The formula of arecaidine was thus resolved into



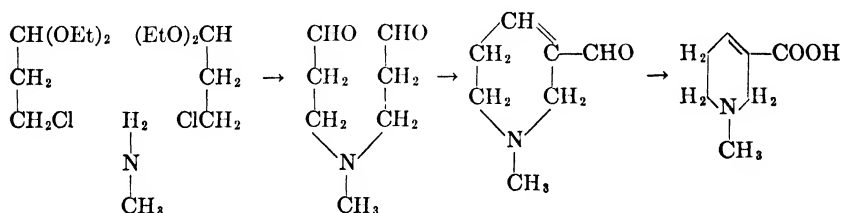
which led Jahns to the conception that it must be a partly saturated pyridine derivative related to nicotinic acid. This theory was confirmed by synthesis;⁴⁴ nicotinic acid methochloride, on reduction with tin and hydrochloric acid, yielded arecaidine.



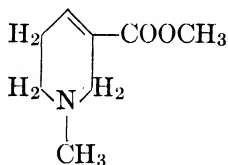
⁴³ Lewin, "Phantastica. Narcotic and Stimulating Drugs," Dutton, New York (1931).

⁴⁴ Jahns, *Ber.*, **21**, 3404 (1888); **23**, 2972 (1890); **24**, 2615 (1891); *Arch. Pharm.*, **229**, 669 (1891).

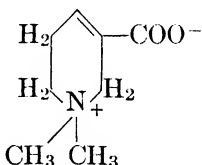
The optical inactivity of arecaidine leaves only the 2,3- and 3,4-positions in question for the double linkage. This uncertainty was eliminated by the synthesis of Wohl.⁴⁵ Acrolein was converted by the action of alcohol and hydrogen chloride into β -chloropropionaldehyde acetal, and two molecules of this product were condensed with methylamine. The resulting methylaminodipropionaldehyde diacetal gave on hydrolysis the dialdehyde, which underwent ring closure with loss of water, yielding the aldehyde corresponding to arecaidine. This was transformed through the oxime and nitrile to the acid, arecaidine.



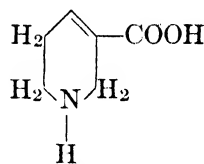
Arecaidine can also be considered as the tetrahydro derivative of the betaine type, trigonelline (nicotinic acid methyl betaine). For arecoline, the corresponding quaternary ammonium formula (IIIa) is in better accord with the physiological action than the ester formula (III).



III. Arecoline



IIIa



IV. Guvacine

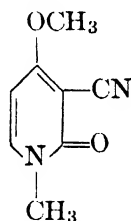
Guvacine (norarecaidine) and guvacoline (norarecoline) are minor alkaloids, related as acid and methyl ester. The constitution of the pair is evident from the identity of guvacine with 1,2,5,6-tetrahydronicotinic acid, further from the conversion of guvacine into arecaidine by N-methylation.

Arecoline stimulates salivation and perspiration; in larger doses it kills by respiratory paralysis. Areca nut extract, as well as arecoline, has vermifugal action and is used for this effect in veterinary medicine. Betel chewers, nevertheless, are often afflicted with intestinal parasites; the alkaloids probably reach the intestinal tract in too low concentration to be effective.

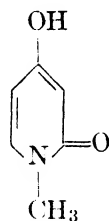
⁴⁵ Wohl and Johnson, *Ber.*, **40**, 4712 (1907).

Castor-Bean Alkaloid. Ricinine, $C_8H_8O_2N_2$, occurs in the seeds and especially in the young plants of *Ricinus communis* (castor-oil plant); it is one of the few alkaloids that is found unaccompanied by others. Ricinine is optically inactive and so weakly basic that it forms no salts.

When ricinine is distilled with zinc dust, pyridine is obtained; catalytic reduction, on the other hand, proceeds with addition of four hydrogen atoms (tetrahydroricinine), facts that point to the presence of a dihydrogenated pyridine nucleus in ricinine. On treatment with alkali, ricinine yields methyl alcohol and the compound $C_7H_6O_2N_2$, which was named ricinic acid (III) in the belief that ricinine was its methyl ester. With fuming hydrochloric acid at 150° ricinine (like-wise ricinic acid) gives carbon dioxide, ammonia, and the base $C_6H_7O_2N$, which Späth⁴⁶ showed by synthesis to be 4-hydroxy-1-methyl-2-pyridone (II).

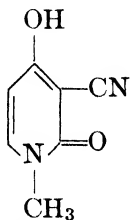


I. Ricinine

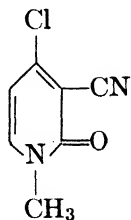


II. 4-Hydroxy-1-methyl-2-pyridone

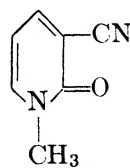
The ricinine structural problem was solved by a study of ricinidine (V), a product obtained by chlorination of ricinic acid with phosphorus oxychloride, and reductive elimination of chlorine. It was found that ricinidine, $C_7H_6ON_2$, could be hydrolyzed in two steps. The addition of one molecule of water gave an amide, $C_7H_8O_2N_2$ (VI), which in the second stage of hydrolysis lost ammonia and was transformed to a carboxylic acid (VII).



III. Ricinic acid



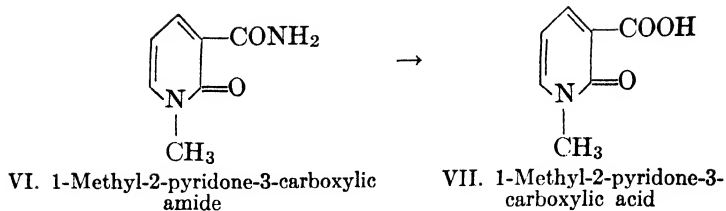
IV. Chlororicinidine



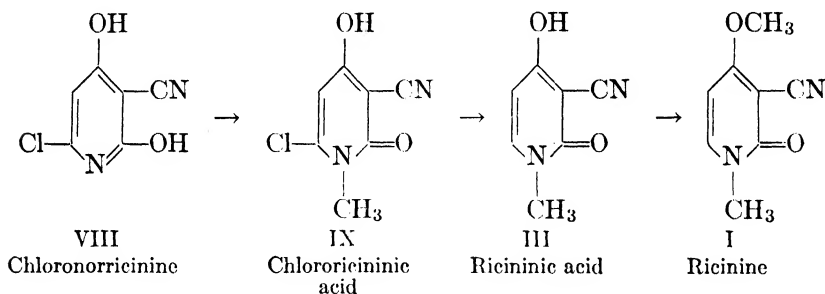
V. Ricinidine



⁴⁶ Späth and Tschelnitz, *Monatsh.*, **42**, 251 (1921).



The structure of the 1-methyl-2-pyridone-3-carboxylic acid so obtained was demonstrated by synthesizing the three possible isomers. The formula thus derived for ricinine was confirmed by Späth's synthesis of the alkaloid itself.⁴⁷ A simple synthetic procedure devised by Schroeter⁴⁸ is based on the observation that spontaneous polymerization of cyanacetyl chloride results in 2,4-dihydroxy-6-chloronicotinic acid nitrile (VIII). On methylation, this substance reacts in a pyridone form, yielding an N-methyl derivative (IX); from the latter, by dehalogenation (formation of ricininic acid III) and further methylation, ricinine is obtained.



Ricinine is mildly poisonous, but the toxic properties of castor beans appear to be due to a phytotoxin, ricin, of unknown nature.

PYRROLIDINE GROUP

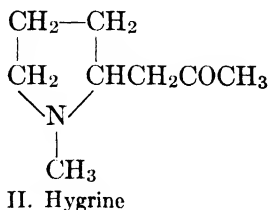
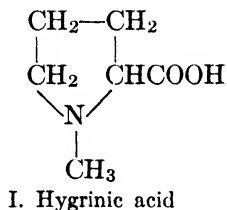
Hygrine Alkaloids. Hygrine, C_8H_5ON , occurs in the leaves of the Peruvian coca shrub, *Erythroxylon coca*, from which it is obtained as an oily fraction along with some cuscohygrine, after the alkaloids of the cocaine group have been removed. Hygrine is one of the liquid alkaloids, and is optically active. The nitrogen atom carries a methyl group, and oxygen is present in ketone form.

The structural formula of hygrine is based upon the relationship to hygrinic (hygric) acid and upon synthesis. By oxidation with chromic

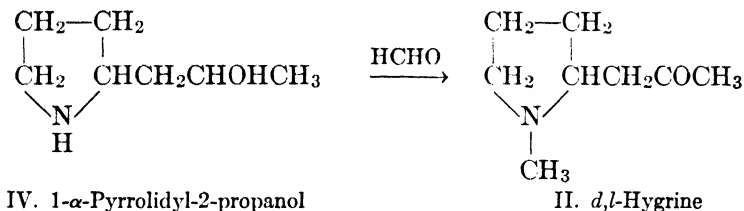
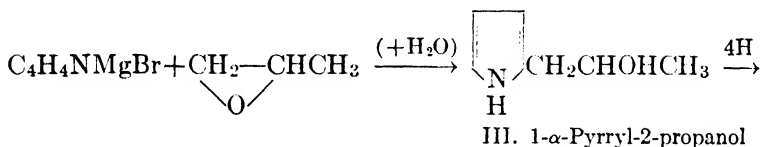
⁴⁷ Späth and Koller, *Ber.*, **56**, 880, 2454 (1923); **58**, 2124 (1925).

⁴⁸ Schroeter, Seidler, Sulzbacher, and Kanitz, *Ber.*, **65**, 432 (1932).

acid, hygrine is converted to hygrinic acid, $C_5H_{10}NCOOH$, a monobasic acid that breaks down on dry distillation into carbon dioxide and N-methylpyrrolidine. The ease of decomposition indicates the α -position for the carboxyl group; this was shown by Willstätter's hygrinic acid synthesis⁴⁹ to be correct.



Hygrine differs from hygrinic acid by a C_3H_5O group in place of carboxyl. For this group only the forms $-\text{COC}_2\text{H}_5$ and $-\text{CH}_2\text{COCH}_3$ are possible; the choice of the latter rests on the (racemic) hygrine synthesis of Hess.⁵⁰ Pyrrolmagnesium bromide was treated with propylene oxide, yielding 1- α -pyrrol-2-propanol, from which the corresponding pyrrolidine derivative (IV) was obtained by catalytic hydrogenation.



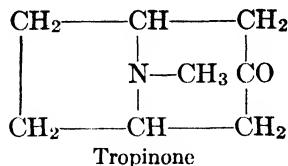
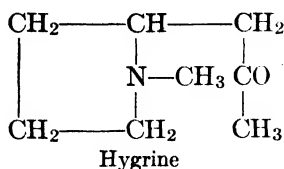
The pyrrolidine imino group was methylated with formaldehyde by the Eschweiler reaction, whereby the secondary alcoholic group unexpectedly contributed its hydrogen atoms toward formation of the methyl group, and appeared as the hygrine carbonyl in the end product.*

⁴⁹ Willstätter, *Ber.*, **33**, 1160 (1900).

⁵⁰ Hess, *Ber.*, **46**, 3113, 4104 (1913).

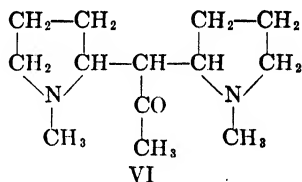
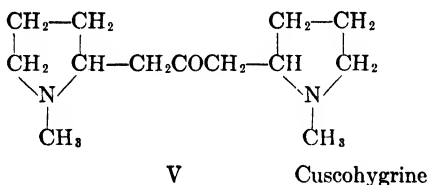
* The Eschweiler method for the methylation of primary or secondary amines consists in heating the amine with formaldehyde. The hydrogen necessary for the formation of the methyl group is supplied by the excess formaldehyde, which is oxidized to formic acid or to carbon dioxide. [Eschweiler, *Ber.*, **38**, 880 (1905); Hess, *Ber.*, **46**, 4104 footnote (1913)].

Attachment of the hygrine side chain to the α -carbon atom of the pyrrolidine nucleus suggests a phytochemical relationship between hygrine and tropinone.⁴⁹



Stachydrine is the methylbetaine of hygrinic acid and occurs rather widely in nature (chrysanthemum, alfalfa, citrus, and *Stachys* varieties).

Cuscohygrine, $\text{C}_{13}\text{H}_{24}\text{ON}_2$, is found chiefly in the so-called cusco coca leaves. It is an optically inactive diacid base, closely related to hygrine. The action of alcoholic alkali degrades it in part to hygrine, and like hygrine it can be oxidized to hygrinic acid. Two formulas have been proposed for cuscohygrine:



Formula V is in better accord with the formation of undecane and 6-undecanol in the Hofmann degradation of dihydrocuscohygrine (i.e., cuscohygrine reduced at the carbonyl group), which indicates an unbranched chain of eleven carbon atoms. Formula VI was advanced by Hess to explain the appearance of homohygrinic acid (N-methyl- α -pyrrolidylacetic acid) in Traube's reaction* and of a supposed di-(N-methyl- α -pyrrolidyl)-methane in decompositions of cuscohygrine hydrazone.⁵¹ Barring the possibility of a rearrangement during the Hofmann degradation, formula V seems the more probable for cuscohygrine.

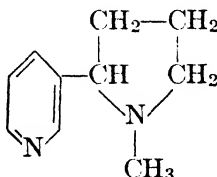
* Traube's reaction [*Ann.*, **300**, 81 (1898)] depends upon the ability of the hydrogen in methyl, methylene, or methenyl groups adjacent to a carbonyl group to react with nitric oxide in the presence of sodium ethoxide. From the number of moles of nitric oxide which react, and the nature of the products of subsequent hydrolysis, it is possible to distinguish between $-\text{COCH}_3$, $-\text{COCH}_2-$, and $-\text{COCH}=-$ groups.

⁵¹ Hess and Bappert, *Ann.*, **441**, 137 (1925); Sohl and Shriner, *J. Am. Chem. Soc.*, **55**, 3828 (1933); Hess and Fink, *Ber.*, **53**, 781 (1920).

PYRIDINE-PYRROLIDINE, DIPYRIDINE GROUP

Tobacco and Anabasis Alkaloids. The alkaloid nicotine, from *Nicotiana tabacum*, occupies a position of great commercial importance. The annual world production of tobacco for human consumption and insecticidal use is more than two million tons, corresponding to about sixty or seventy thousand tons of nicotine alkaloid. The base is combined in the plant with malic and citric acids and may be isolated by extracting the powdered leaves and stems with water, liberating the alkaloids with alkali, and distilling with steam. The crude nicotine is purified through the oxalate. It is also commercial practice to extract systematically with trichloroethylene a mixture of tobacco refuse, milk of lime, and sodium hydroxide. The solution is concentrated in a vacuum and extracted with dilute sulfuric acid, from which the nicotine is liberated with alkali and extracted into ether-petroleum ether mixture. Distillation of this solution under nitrogen yields nearly pure nicotine.

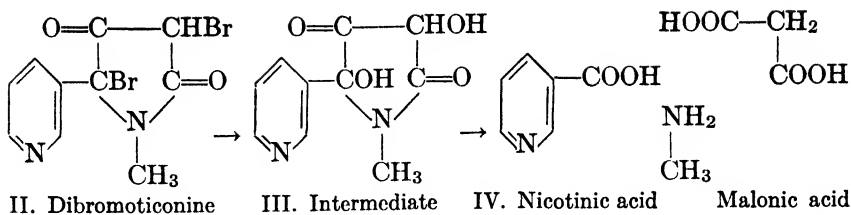
Nicotine is a strongly basic levorotatory liquid, miscible with water below 60° and above 210° in consequence of hydrate formation. Its structure has been shown both by degradation and synthesis. Oxidation with a variety of agents leads to nicotinic acid, β -pyridinecarboxylic acid (IV). The alkaloid must therefore be a pyridine derivative carrying a $C_5H_{10}N$ group in the β -position. This side chain cannot consist of a piperidine nucleus, for nicotine behaves as a bi-tertiary base, that is, contains no $>NH$ group; furthermore, the Herzig and Meyer determination shows the presence of an N-methyl group. The methyl group cannot be attached to the pyridine nitrogen atom, so the $C_5H_{10}N$ group is resolved into $C_4H_7NCH_3$. These facts are best explained by a pyridine-N-methylpyrrolidine system.⁵²



I. Nicotine

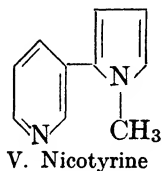
The linkage of the pyridine ring in the α -position of the pyrrolidine nucleus was shown by bromine degradation. Nicotine, on treatment with bromine, yields a dibromoketone, dibromoticonine. With barium hydroxide, dibromoticonine breaks down to nicotinic acid, malonic acid, and methylamine.

⁵² Pinner, *Ber.*, **26**, 292 (1893).

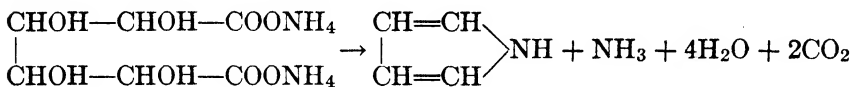


The appearance of the three-carbon acid, malonic, shows that the carbon atom appearing in the carboxyl group of nicotinic acid must be the end atom of a chain of four carbons, which is possible only if the pyrrolidine ring is linked through an α -position.

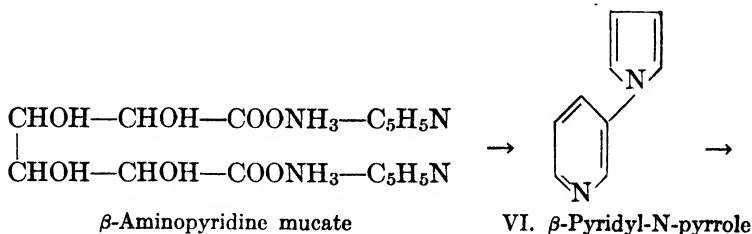
With weak oxidizing agents the methylpyrrolidine nucleus of nicotine is attacked, resulting in nicotyrine, a base that appears as an intermedi-



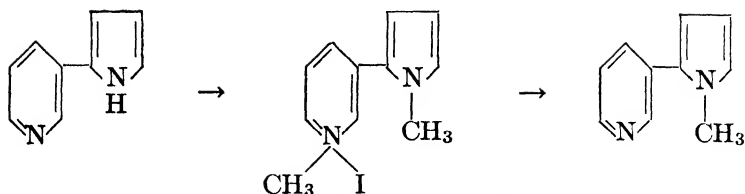
ate in the nicotine synthesis of Pictet.⁵³ This synthesis has its starting point in a reaction parallel to the formation of pyrrole through dry distillation of ammonium mucate.



By using the mucate of β -aminopyridine, that is, by substituting the pyridine group for one hydrogen of ammonia in the above reaction, Pictet obtained β -pyridyl-N-pyrrole. Pyrroles carrying carbon substituents on nitrogen undergo on heating a rearrangement that involves a shift of the group from nitrogen to the pyrrole α -position, and β -pyridyl-N-pyrrole was converted by this method to β -pyridyl- α -pyrrole.



⁵³ Pictet and Rotschy, *Ber.*, **37**, 1225 (1904).

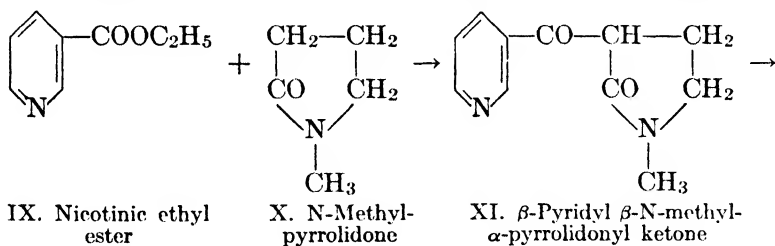
VII. β -Pyridyl- α -pyrrole

VIII. Nicotyrine methiodide

V. Nicotyrine

The potassium salt of the latter substance, when heated with methyl iodide, yielded nicotyrine methiodide, from which nicotyrine (V) could be obtained by heating with lime. By halogenation and reduction with tin and acid, the methylpyrrol nucleus alone of nicotyrine was hydrogenated, and the resulting *d,l*-nicotine could be resolved with tartaric acid into *d*-nicotine and the familiar *l*-nicotine. The reduction of nicotyrine can also be accomplished with catalytic hydrogen.⁵⁴

The Pictet synthesis involves violent and complicated reactions which are of doubtful value for structural proof. A more transparent synthesis by Späth⁵⁵ confirms, however, the accepted structure. Nicotinic ethyl ester was condensed with *N*-methylpyrrolidone, resulting in the ketone (XI). The pyrrolidonyl ring of this ketone suffered scission and loss of carbon dioxide when heated with fuming hydrochloric acid, yielding the open-chain amino ketone (XII). By reduction of the amino ketone to the corresponding alcohol, iodination, and elimination of hydrogen iodide, the *N*-methylpyrrolidine ring of nicotine was constructed in a manner that leaves no question as to its point of attachment.



IX. Nicotinic ethyl ester

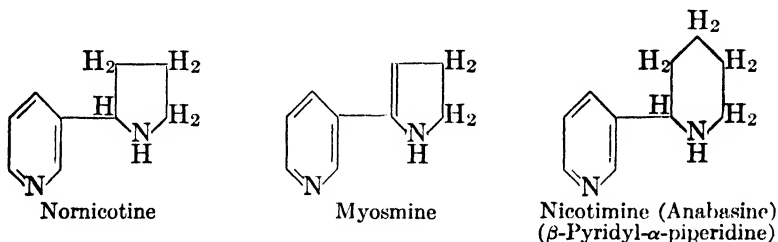
X. *N*-MethylpyrrolidoneXI. β -Pyridyl β -*N*-methyl- α -pyrrolidonyl ketoneXII. β -Pyridyl γ -methyl-amino-*n*-propyl ketone

I. Nicotine

⁵⁴ Späth and Kuffner, *Ber.*, **68**, 494 (1935).⁵⁵ Späth and Bretschneider, *Ber.*, **61**, 327 (1928).

The synthesis of α -nicotine, α -pyridyl- α -N-methylpyrrolidine, which is not known to occur naturally, has also been accomplished.⁵⁶

Pictet and Noga have described nicotine, isonicotine, nicotine, nicotimine, and nicotelline as minor alkaloids. The most abundant of these, nicotine, was shown by Ehrenstein⁵⁷ to be a mixture of two alkaloids, so that the existence of the rarer members as individuals may well be doubted. The so-called nicotine was separated by fractional crystallization of the picrate into nornicotine and *l*- β -pyridyl- α -piperidine. The latter substance has the formula that had already been assigned without adequate evidence to Pictet's nicotimine. Nornicotine can be prepared by demethylation of nicotine, or by total synthesis from pyridine.^{58,59} Both *d*- and *l*-nornicotine have been found in tobacco. The constituents of tobacco smoke have been extensively studied. There appear to be at least eight bases present, of which myosmine and the three sokratines are responsible for the aroma. Myosmine has been shown to be a β -pyridyl- α -pyrroline.⁶⁰



As the chief alkaloid of the poisonous Asiatic plant *Anabasis aphylla*, Orechoff⁶¹ isolated the base anabesine. This substance is identical with the above-mentioned *l*- β -pyridyl- α -piperidine. Its constitution could be shown by oxidation to nicotinic acid, and by dehydrogenation to α,β -bipyridyl.

Nicotine is one of the most poisonous alkaloids, the fatal dose for man being in the neighborhood of 40 mg. In smaller amounts it causes dizziness, perspiration, salivation, and intestinal disturbances. *d*-Nicotine shows only one-half the physiological activity of natural *l*-nicotine. Anabesine, like nicotine, is very poisonous and has high insecticidal action.

⁵⁶ Craig, *J. Am. Chem. Soc.*, **56**, 1144 (1934).

⁵⁷ Ehrenstein, *Arch. Pharm.*, **269**, 627 (1931).

⁵⁸ Craig, *J. Am. Chem. Soc.*, **55**, 2854 (1933).

⁵⁹ Späth, Marion, and Zajic, *Ber.*, **69**, 251 (1936).

⁶⁰ Späth, Wenusch, and Zajic, *Ber.*, **69**, 393 (1936); Späth and Mamoli, *Ber.*, **69**, 757 (1936).

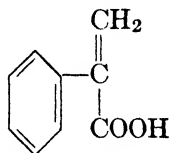
⁶¹ Orechoff and Menschikoff, *Ber.*, **64**, 266 (1931).

**CONDENSED PIPERIDINE-PYRROLIDINE GROUP. BELLADONNA
AND COCA ALKALOIDS**

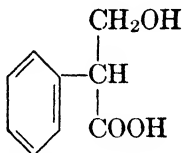
Belladonna Alkaloids. The roots and leaves of a number of solanaceous plants, notably belladonna (*Atropa belladonna*), henbane (*Hyoscyamus niger*), the thorn apple (*Datura stramonium*), and some *Duboisia* and *Scopolia* varieties, are rich in a series of therapeutically important alkaloids. Hyoscyne and hyoscyamine occur in nearly all these plants, accompanied occasionally by atropine, apoatropine, norhyoscyamine, belladonnine, and meteloidine. The solanaceous plants are notorious hallucinants, the drugs of fanaticism. The group furnished the "sorcerer's drugs" of the Middle Ages, and *Hyoscyamus*, *Datura*, and *Duboisia* varieties are today smoked, chewed, or consumed in decoctions in parts of Egypt, India, South America, and Australia for the hallucinations and frenzy that they produce.⁴³

Atropine, $C_{17}H_{23}O_3N$, is the racemic form of hyoscyamine. Although it is undoubtedly formed to a large extent from the latter base during isolation and purification, it has also been shown to exist as such in the plant. All the atropine of commerce is prepared by racemization of hyoscyamine with dilute alkali. Atropine is an ester; on hydrolysis it yields tropic acid, $C_9H_{10}O_3$, and tropine, $C_8H_{15}ON$.

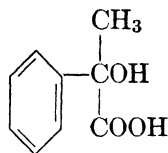
Determination of the structure of tropic acid offered little difficulty. This acid is converted by dehydrating agents to α -phenylacrylic acid (atropic acid), a type of change characteristic of β -hydroxy acids, but not of α -hydroxy acids. Tropic acid must therefore possess formula II, for the only alternative (III) is that shown by synthesis to belong to atrolactic acid.



I. Atropic acid



II. Tropic acid



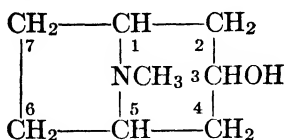
III. Atrolactic acid

Tropic acid was synthesized by Ladenburg from acetophenone.⁶² It contains an asymmetric carbon atom, and to it hyoscyamine owes its optical activity.

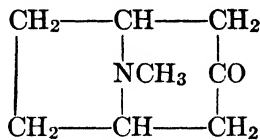
The basic portion obtained from the hydrolysis of atropine or hyoscyamine, namely tropine, is optically inactive. The two asymmetric carbon atoms in positions 1- and 5- compensate, and the 3-carbon atom is pseudoasymmetric. The molecule is symmetrical, and cannot be

⁶² Ladenburg and co-workers, *Ber.*, **13**, 2041 (1880); **22**, 2590 (1889).

resolved into active components. The tropine structural formula was developed chiefly by Merling and by Willstätter on the basis of the following evidence. Tropine is a tertiary base containing an N-methyl group and an alcoholic hydroxyl. By gentle oxidation it is converted to a ketone, tropinone.

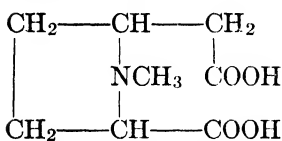


IV. Tropine

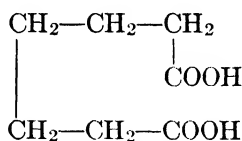


V. Tropinone

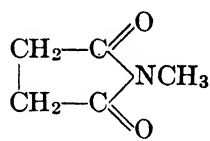
This ketone forms diisonitroso and dibenzylidene derivatives, and therefore has two methylene groups adjacent to the carbonyl. Tropinone gives on further oxidation a dicarboxylic acid (tropinic acid, VI) with the same number of carbon atoms, hence the ketone group cannot be in a side chain. Application of the exhaustive methylation process to tropinic acid yields pimelic acid (compare the degradation of methylgranatic acid, p. 1034) containing a straight seven-carbon chain. Oxidation of tropinic acid, on the other hand, results in N-methylsuccinimide, whereby the position of nitrogen is shown, and the pyrrolidine ring is revealed.



VI. Tropinic acid

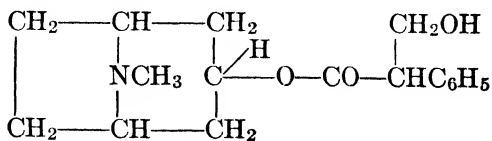


VII. Pimelic acid



VIII. N-Methylsuccinimide

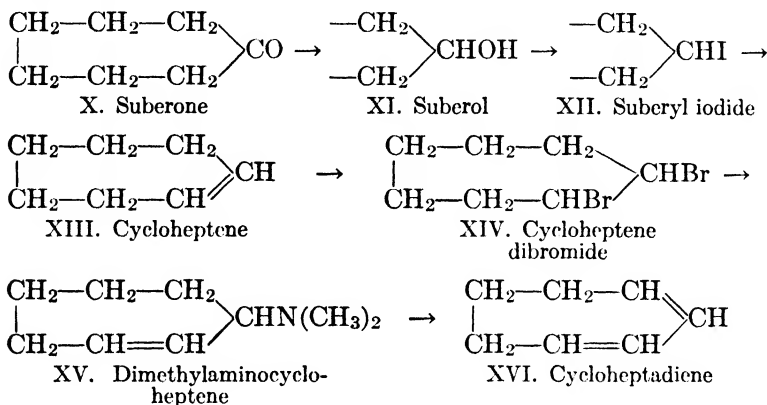
Tropine consists therefore of a fused piperidine-pyrrolidine skeleton in which the two ring systems have nitrogen and two carbon atoms in common. The esters of tropine, of which many have been prepared, are called tropeines; the most important synthetic ester is mandelyltropine, a powerful mydriatic known as homatropine. Atropine is *d,l*-tropyltropine, hyoscyamine is the *levo* form.



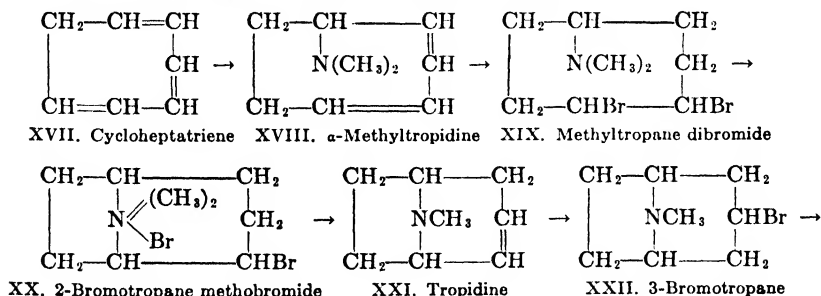
IX. Atropine, Hyoscyamine

The preparation of atropine from tropine and tropic acid was accom-

plished by Ladenburg in 1879,⁶³ and both components were later synthesized. For the preparation of tropine, Willstätter⁶⁴ chose suberone as the starting point. This ketone was converted to cycloheptene through suberol and suberyl iodide, or by exhaustive methylation of the amine resulting from reduction of suberone oxime.

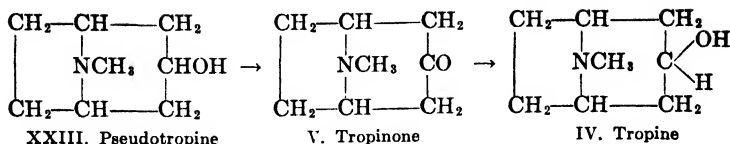


A second unsaturated linkage was introduced by the following ingenious device. Cycloheptene dibromide was treated with dimethylamine, yielding a tertiary amine, the methiodide of which could be degraded by Hofmann's method (p. 1024) to trimethylamine and cycloheptadiene. Cycloheptadiene dibromide suffered loss of hydrobromic acid in the presence of quinoline to give cycloheptatriene (XVII), identical with the tropilidene already known from the degradation of tropine. By addition of hydrogen bromide and subsequent reaction with dimethylamine, cycloheptatriene was converted to the amine, α -methyltropidine. Partial reduction of α -methyltropidine, addition of bromine, and rearrangement of the dibromo compound led to 2-bromotropane methobromide, containing the desired nitrogen bridge.



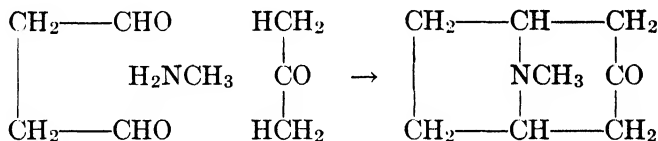
⁶³ Ladenburg, *Ber.*, **12**, 941 (1879).

⁶⁴ Willstätter, *Ber.*, **34**, 131, 3163 (1901); *Ann.*, **326**, 23 (1903).



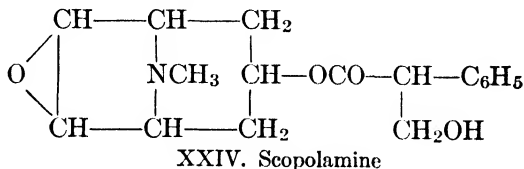
From 2-bromotropane methobromide, hydrogen bromide was eliminated by the action of alkali, giving tropidine methobromide. Through the usual steps for converting a quaternary halide to the tertiary base, tropine methobromide was transformed to the methochloride and the latter distilled; tropidine (XXI) and methyl chloride were the products. From tropidine and hydrobromic acid, 3-bromotropane was obtained, which on hydrolysis with dilute sulfuric acid yielded instead of the expected tropine, the stereoisomer, pseudotropine. Pseudotropine was therefore oxidized to tropinone, which could then be reduced to tropine.

Another synthesis of extraordinary simplicity and elegance was devised by Robinson.⁶⁵ Succinaldehyde, methylamine, and acetone (or better, calcium acetonedicarboxylate), on standing in alkaline solution gave tropinone.



A later synthesis by Willstätter likewise has its starting point in acetone-dicarboxylic acid.⁶⁶ The success of Schöpf in carrying out the Robinson synthesis under physiological conditions (p. 1108) makes it seem probable that the plant employs a similar method.

Scopolamine, also known as hyoscyne, is an ester of the optically inactive amino alcohol scopine (XXV) with *l*-tropic acid.



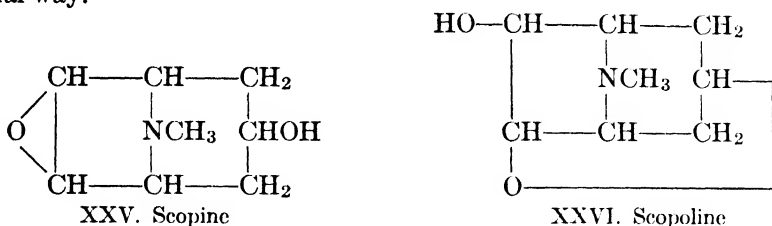
Scopolamine is levorotatory, but is racemized with great ease, the *d,l*-form being atropine. By hydrolysis of scopolamine under very mild conditions, with pancreatic lipase or with Michaelis' buffer solution, Willstätter,⁶⁷ was able to obtain scopine itself (XXV). The latter

⁶⁵ Robinson, *J. Chem. Soc.*, **111**, 762 (1917).

⁶⁶ Willstätter and Pfannenstiel, *Ann.*, **422**, 1 (1921).

⁶⁷ Willstätter and Berner, *Ber.*, **56**, 1079 (1923).

undergoes rearrangement with great ease into scopoline (oscine), which is the basic product obtained when scopolamine is hydrolyzed in the usual way.

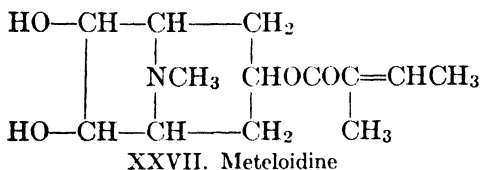


Apoatropine (atropamine), found in belladonna root, can also be obtained from atropine or hyoscyamine by the action of dehydrating agents. It is an ester of tropine and atropic acid (α -phenylacrylic acid, I), and was obtained by combining these two substances before it was found in nature.

Belladonnine is an isomer or polymer of apoatropine and was first isolated from belladonna root. It can be prepared by the action of hot baryta water on apoatropine. By vigorous hydrolysis with hydrochloric acid it can be broken down to 3-chlorotropane, showing that it contains the tropine nucleus.⁶⁸

Norhyoscyamine (pseudohyoscyamine), from *Duboisia*, *Scopolia*, and *Datura* varieties consists of tropic acid esterified with nortropine (tropigenine), a tropine containing the $>\text{NH}$ group in place of $>\text{NCH}_3$. Norhyoscyamine can be racemized easily to the corresponding *d,l*-form, noratropine. The latter probably does not occur in nature but appears as a result of racemization of the active form.

Meteloidine is a rare alkaloid of *Datura meteloides* and is an ester of tiglic acid with teloidine. Teloidine is believed to be dihydroxytropine and is closely related to scopine and scopoline.⁶⁹



The alkaloids of the atropine group dilate the pupil and paralyze the accommodation muscles of the eye. Atropine thus finds extensive use in ophthalmic practice. It has a stimulating action on the cerebrum and respiratory center. Hyoscyamine resembles atropine, but is

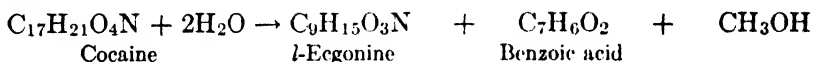
⁶⁸ Polonovski, *Bull. soc. chim.*, [4] **45**, 304 (1929).

⁶⁹ King, *J. Chem. Soc.*, **115**, 476 (1919).

stronger in action. Scopolamine has a stupefying effect, and is often used in combination with morphine, as well as in the treatment of morphinism.

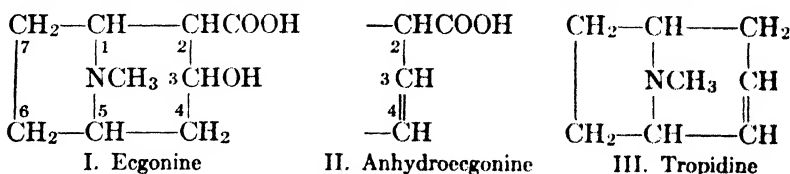
Coca Alkaloids. The leaves of *Erythroxylon coca*, which have been used as a stimulant by the South American Indians for centuries, contain as the active principle cocaine, with which is associated a number of other alkaloids of closely related structure. The great importance of cocaine in medical practice has resulted in extensive cultivation of several *Erythroxylon* species in Peru, Bolivia, Java, and Ceylon. The legitimate world production of cocaine has dropped from 6434 kg. in 1929 to 4010 kg. in 1933, probably because of increasing use of substitutes and more effective control of international trade. The illegitimate production is large, and is unofficially estimated at 15,000 to 20,000 kg.

Cocaine, $C_{17}H_{21}O_4N$, is an ester and is hydrolyzed by boiling water into benzoyl-*l*-ecgonine and methanol, or by acids and alkalis into *l*-ecgonine, benzoic acid, and methanol.



This process may be reversed, and in commercial practice it is customary, especially with Java leaves, to hydrolyze all the ecgonine derivatives present (including the cocaine) to ecgonine. This base is then benzoylated with benzoic anhydride and the benzoyl-ecgonine esterified with methanol and acid; methylation followed by benzylation is also employed. An amount of cocaine considerably greater than that originally present in the leaves is thus obtained.

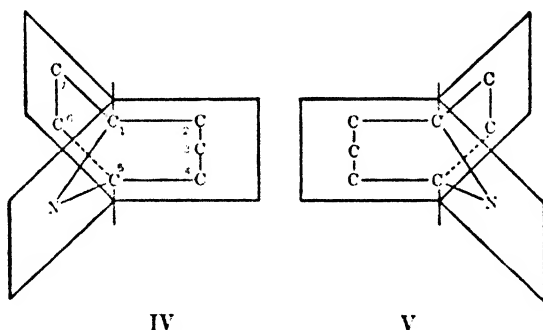
Ecgonine is a tertiary base and forms quaternary halides with one mole of alkyl halide. The presence of the carboxyl and alcoholic hydroxyl groups is evident from the esterification reactions mentioned. The structural skeleton of ecgonine was disclosed through relationships to tropine. With dehydrating agents ecgonine passes into anhydroecgonine, an unsaturated acid, which decomposes in the presence of hydrochloric acid at 280° into carbon dioxide and tropidine. The constitution of tropidine is discussed under the atropine group.



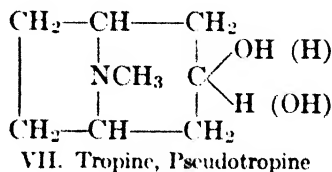
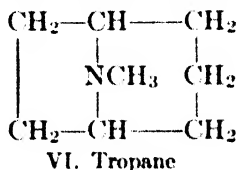
The position of the hydroxyl and carboxyl groups of ecgonine rests on the following considerations. Ecgonine, like tropine, yields by

chromic acid oxidation tropinone, and since the carbonyl can scarcely be formed except by oxidation of the alcoholic group, ecgonine must have the hydroxyl in the same position as has tropine. Willstätter⁷⁰ was able to demonstrate that the oxidation of ecgonine proceeds through an intermediate keto acid, which loses carbon dioxide with great ease. The appearance of the keto acid excludes the possibility that the carboxyl and hydroxyl groups occupy the same carbon atom. Location of the carboxyl in a γ -position to the hydroxyl is not in accord with the instability of the intermediate acid, hence only a β -position comes into consideration.

Tropane, the parent substance of the ecgonine series, contains two asymmetric carbon atoms, C-1 and C-5, to which the nitrogen bridge is attached. These asymmetric atoms are equal and opposite in their rotatory power, which results in internal compensation. Tropane is a meso-form, and has a symmetrical molecule.



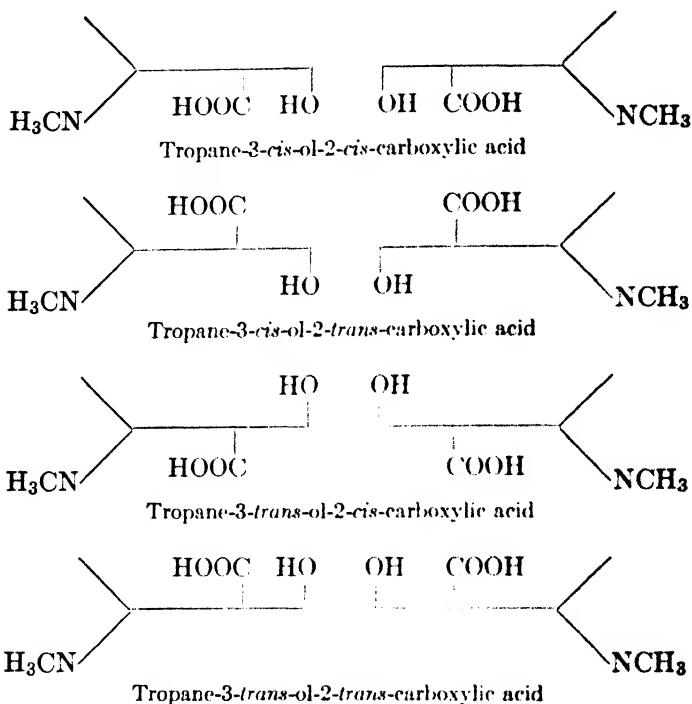
When a hydroxyl group appears on C-3 (as in tropine), the symmetry of the molecule is not destroyed; C-3 in tropine is pseudoasymmetric. The hydroxyl may occupy two positions with reference to the nitrogen ring, giving rise to tropine and pseudotropine, which are *cis-trans* isomers and not optical opposites.



The presence of a carboxyl group in the tropine or pseudotropine framework destroys the symmetry of the molecule. In addition to the new asymmetric atom carrying the carboxyl, carbon-3 now becomes

⁷⁰ Willstätter and Müller, *Ber.*, **31**, 2655 (1898).

truly asymmetric, and the asymmetric atoms 1- and 5-, carrying the nitrogen bridge, become dissimilar. Sixteen optically active isomers would be expected, but C-1 and C-5 can have only one configuration because of the restriction imposed by the nitrogen bridge. Therefore only eight optical isomers and four racemates can exist.^{71,72} A vertical projection of the three tropane ring planes of Figs. IV and V shows these isomers thus:

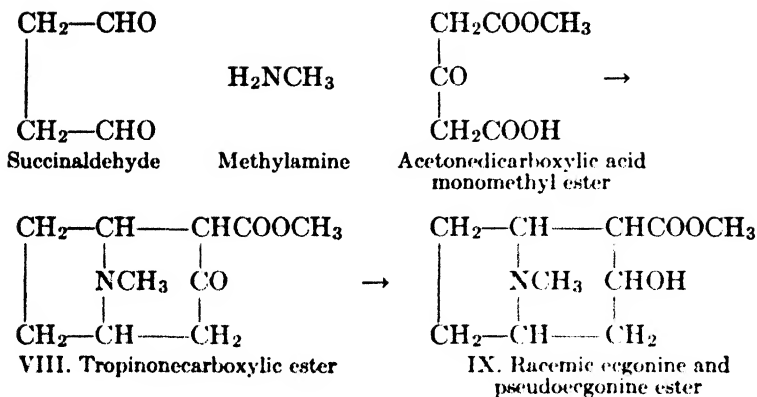


The synthesis of ecgonine through reduction of tropinonecarboxylic ester (VIII to IX) led to the isolation of a racemic ecgonine methyl ester belonging to the pseudo series. On resolution the racemate yielded *d*-pseudoecgonine methyl ester and the corresponding *l*-form, from which *d*-pseudococaine (the drug Psicain) and *l*-pseudococaine, respectively, could be prepared by benzylation. In the same reduction two other racemates were formed. One of these has not been resolved. The other belongs to the tropine series and after benzylation was resolved into *d*-cocaine and the naturally occurring *l*-cocaine.⁷³

⁷¹ Willstätter and Bommer, *Ann.*, **422**, 15 (1921).

⁷² Mannich, *Arch. Pharm.*, **272**, 324 (1934).

⁷³ Willstätter, Wolfes, and Mäder, *Ann.*, **434**, 111 (1923).



α -Egonine is an isomer of ecgonine in which both the hydroxyl and carboxyl groups are located on carbon-3. It was prepared by addition of hydrogen cyanide to tropinone and hydrolysis of the resulting cyanohydrin.

l-Cinnamylcocaine is the ester of methyl-*l*-ecgonine with cinnamic acid. It is the chief alkaloid of Java coca leaves (*Erythroxylon truxillense*).

α -Truxilline, also known as cocaine or γ -isatropylcocaine, is an ester of two molecules of methyl-*l*-ecgonine with one molecule of α -truxillic acid. β -Truxilline (isococaine or δ -isatropylcocaine) is the analogous ester with β -truxillic acid. Both truxillines are present in Peruvian leaves.

Tropacocaine, which occurs in Java and Peruvian leaves, does not belong to the ecgonine series, but is a tropa alkaloid. On hydrolysis it yields benzoic acid and pseudotropine. In addition to the above-mentioned alkaloids, coca also contains small amounts of benzoyl-*l*-ecgonine, and the hygrine alkaloids, which have already been described.

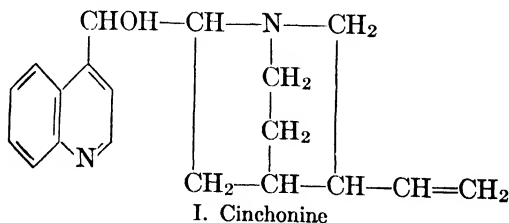
Cocaine is an exceedingly valuable therapeutic agent because of its paralyzing effect on sensory nerve endings, with which is combined a local vasoconstriction. The latter action results in prolongation of the anesthesia by diminishing the speed of absorption; the delayed absorption likewise decreases systemic toxicity by permitting gradual destruction of the drug. Cocaine causes dilation of the pupils by central and peripheral stimulation of the pupillo-dilator mechanism. The relatively high toxicity of cocaine and its ability to produce a condition of euphoria, often leading to habituation, have resulted in the synthesis of numerous substitutes, as novocaine (the *p*-aminobenzoyl derivative of diethylaminoethanol), β -eucaine (benzoylvinyl diacetone alkamine hydrochloride), and Psicain (*d*-pseudococaine acid tartrate). Tropacocaine is said to

be more effective than cocaine as a local anesthetic, but has a disadvantageous hyperemic action.

QUINOLINE GROUP. CINCHONA AND ANGOSTURA ALKALOIDS

Cinchona Alkaloids.⁷⁴ Quinine and cinchonine, together with some twenty less important alkaloids of related structure, are found in the bark of several species of *Cinchona* and *Remijia*, trees native to high altitudes in the Andes. The bark of cultivated specimens of *C. ledgeriana* grafted on *C. succiruba*, as is customary in Java, may contain up to 6 per cent quinine, or total alkaloids up to 17 per cent. The famous *ledgeriana* graft "38n" contained in the trunk bark 18.5 per cent quinine (as sulfate) at the age of seven years. The world production of quinine follows the demand closely, and averages between 600,000 and 700,000 kg. of quinine sulfate annually, 90 per cent of which is from the Dutch East Indies.⁷⁵ The bases are combined in the plant tissue with characteristic acids, chiefly quinic (tetrahydroxyhexahydrobenzoic), quinovic ($C_{30}H_{46}O_5$), and quinotannic (cinchotannic) acids. In commercial practice, the pulverized bark is steeped in slaked lime and sodium hydroxide, and extracted at 60° with aromatic solvents, as benzene or toluene. The mixed alkaloids are then extracted from the organic medium with dilute sulfuric acid. When this solution is brought nearly to the neutral point with sodium hydroxide, the sparingly soluble quinine sulfate, $Q_2H_2SO_4 \cdot 8H_2O$, separates. The less valuable minor alkaloids are then precipitated with excess alkali.

The parent alkaloid of the cinchona series, to which nearly half of the members are related, is cinchonine, $C_{19}H_{22}ON_2$. Cinchonine consists of a quinoline nucleus linked through a secondary alcoholic group to a quinuclidine ring system carrying a vinyl group.

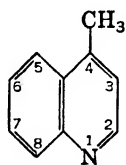


The usual analytical procedure shows the absence of methoxyl and methylimido groups in cinchonine. The presence of the secondary

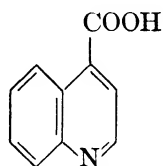
⁷⁴ Comanducci, "Die Konstitution der Chinaalkaloide," in Ahrens' "Samml. chem. chem.-tech. Vorträge," Vol. 16, p. 141 (1911).

⁷⁵ "Chininum," Bureau Tot Bevordung van het Kinine-Gebruik, Amsterdam (1923).

alcoholic group is evident from the results of acetylation and from the formation of the ketone, cinchoninone (VIII), in oxidation processes. The absorption of one mole of hydrogen by the catalytic method shows an ethylenic linkage. Cinchonine likewise adds halogens or halogen acids at the double bond. On treatment with hot concentrated alkali it is broken down to quinoline itself, or lepidine (4-methylquinoline), as well as to other quinoline and pyridine derivatives. Zinc dust distillation yields chiefly quinoline; vigorous oxidation results in cinchoninic acid.

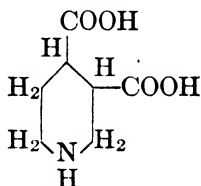


II. Lepidine

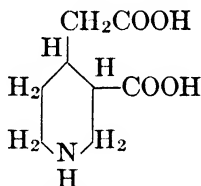


III. Cinchoninic acid

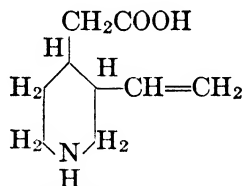
The products of these degradations indicate a quinoline nucleus joined in the 4-position with a second heterocyclic ring. This other ring was designated for many years as "the second half," and substances derived from it were usually distinguished by the use of *loipon* or *meros* in their names. As fragments of the second half, Skraup was able to isolate after chromic acid oxidation the dibasic loiponic ($C_7H_{11}O_4N$) and cincholoiponic ($C_8H_{13}O_4N$) acids, and Koenigs found further a monobasic acid, meroquinene ($C_9H_{15}O_2N$).



IV. Loiponic acid



V. Cincholoiponic acid



VI. Meroquinene

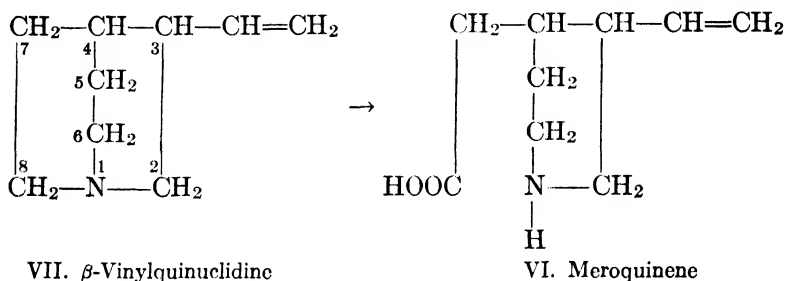
Loiponic acid proved to be a labile form of the synthetically prepared hexahydrocinchomeronic acid (piperidine-3,4-dicarboxylic acid).⁷⁶ Cincholoiponic acid, a homolog of loiponic acid, can be converted by oxidation to loiponic acid, or by the action of hot sulfuric acid to γ -picoline (4-methylpyridine). The structure developed for cincholoiponic acid (V) from these observations was confirmed by Wohl's synthesis.⁷⁷

The third product of cinchonine oxidation, meroquinene, furnished the key to the structure of the second half. On oxidation with perman-

⁷⁶ Koenigs, *Ber.*, **30**, 1326 (1897).

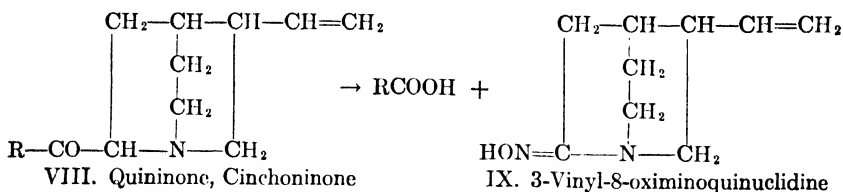
⁷⁷ Wohl and Losanitsch, *Ber.*, **40**, 4698 (1907).

ganate, meroquinene yields cincholoiponic acid and formic acid, or by heating with hydrochloric acid it passes into 3-ethyl-4-methylpyridine. These facts indicate that the vinyl group is in the β -position to the nitrogen atom in the second half. All three oxidation products under discussion are secondary bases. The nitrogen atoms in cinchonine are tertiary, and since no N-methyl groups are present, the nitrogen in the second half must owe its tertiary nature to linkage in a condensed ring system. Oxidation of the second half to meroquinene involves oxidative scission of a ring, with formation of a carboxyl and an imino group.



A quinuclidine ring synthesis leading to β -ethylquinuclidine (which constitutes the second half in the natural alkaloids hydrocinchonine and hydroquinine) was accomplished by Koenigs.⁷⁸

The point of linkage of the quinuclidine group to the rest of the molecule was shown by Rabe⁷⁹ in studies on quinone and cinchoninone, the ketones resulting from gentle oxidation of quinine and cinchonine, respectively. These ketones have a CH group adjacent to, and activated by, the carbonyl group, and on treatment with amyl nitrite break down to quinoline acids (quininic and cinchomeronic, respectively) and an oxime (IX), 3-vinyl-8-oximinoquinuclidine.



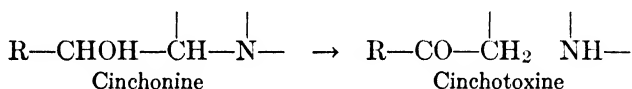
The structure of the latter compound was evident from the results of hydrolysis, which yielded hydroxylamine and meroquinene. The quinuclidine group must therefore be joined to the quinoline portion through a $-\text{CHOH}-$ group attached to that carbon atom which appears as a carboxyl group in meroquinene.

⁷⁸ Koenigs and Bernhart, *Ber.*, **38**, 3049 (1905).

⁷⁹ Rabe, *Ann.*, **365**, 353 (1909); *Ber.*, **41**, 62 (1908).

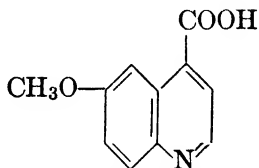
Cinchonine and the other alkaloids of the cinchona group containing a vinyl group may be oxidized to the class designated as "tenines." Cinchotenine has a carboxyl group in place of the β -vinyl residue in cinchonine; from quinine and cupreine, quitenine and cuprotenine are obtained.

The cinchona alkaloids are further characterized by the ease with which they undergo isomerization. Of the seventeen or more cinchonine isomers that have been described by various investigators, at least eight are individuals. The most important of these are cinchotoxine and the natural alkaloid, cinchonidine, which also results from treatment of cinchonine with alkali. Cinchotoxine is a rearrangement product obtained by the action of heat on cinchonine salts; many of the cinchona alkaloids undergo a similar rearrangement to toxines, so called because of their poisonous properties. The isomerism is due to the following change, the so-called "hydramine fission":⁸⁰



The cinchonatoxines can be converted through the cinchona ketones back to the alkaloids, a fact of great importance for synthesis (p. 1060).

Quinine, $\text{C}_{20}\text{H}_{24}\text{O}_2\text{N}_2$, is the most important of the cinchona alkaloids because of its extensive use as a febrifuge and antimalarial. Like cinchonine, quinine contains an alcoholic hydroxyl and a vinyl group, but possesses in addition a methoxyl group. By demethylation with hydrochloric acid it is split to methyl chloride and apoquinine, a phenolic base isomeric with the alkaloid cupreine. In the demethylation process a rearrangement takes place; methylation of apoquinine results in β -isoquinine, an isomer of quinine.⁸¹ The position of the quinine methoxyl group is evident from the appearance of 6-methoxyquinoline-4-carboxylic acid (quininic acid) in oxidations of quinine.



X. Quinic acid

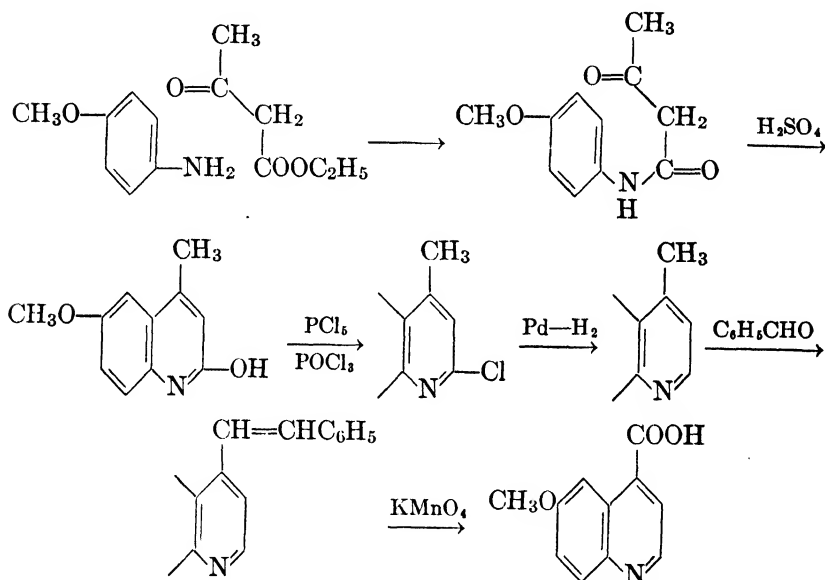
The other products from the oxidation, namely meroquinene, cincholo-

⁸⁰ Rabe and Schneider, *Ann.*, **365**, 377 (1909).

⁸¹ Hesse, *Ann.*, **205**, 322 (1880); *Ber.*, **28**, 1301 (1895); Jarzyński, Ludwiczakówna, and Suszko, *Rec. trav. chim.*, **52**, 839 (1933).

ponic acid, and loiponic acid, show that the quinuclidine portion is the same as in cinchonine; quinine is 6'-methoxycinchonine.

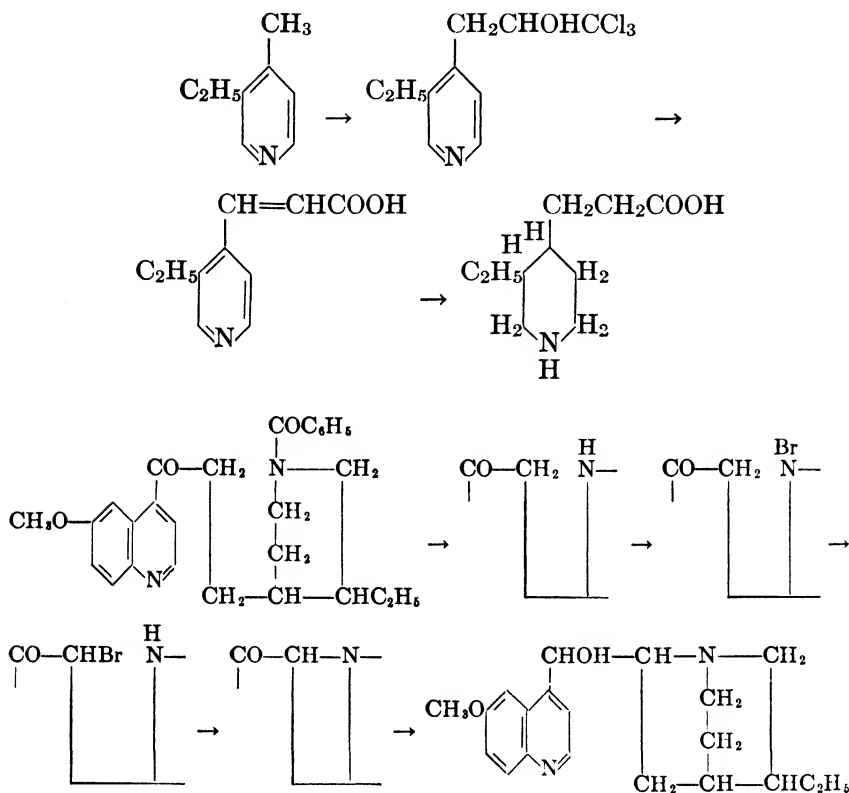
A total synthesis of the complex structure present in the cinchona group has been accomplished by Rabe⁸² in the preparation of the isomeric alkaloids hydroquinine and hydroquinidine. Both of these bases are present in cinchona bark; hydroquinine is formed when quinine is hydrogenated. The quininic acid necessary for the synthesis was prepared by condensation of *p*-anisidine with acetoacetic ester, followed by ring closure, elimination of the phenolic hydroxyl, and oxidation.



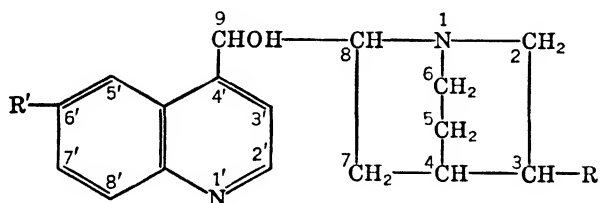
The second necessary constituent, homocincholoipon, was prepared from 3-ethyl-4-methylpyridine (β -collidine). β -Collidine was condensed with chloral and the product converted to ethylpyridylacrylic acid with sodium ethoxide. On hydrogenation, the ethylpyridylacrylic acid gave a mixture of four optically isomeric ethylpiperidylpropionic acids. Resolution of the ethyl esters of these acids with tartaric acid yielded in large part the desired homocincholoipon.

N-Benzoylhomocincholoipon ethyl ester was condensed in the presence of sodium ethoxide with quininic ethyl ester to hydroquinotoxine, and the toxine transformed by bromination into hydroquinidine, which on hydrogenation gave, according to the conditions, hydroquinidine or the stereoisomeric hydroquinine, identical with the natural bases.

⁸² Rabe, Huntenburg, Schultze, and Volger, *Ber.*, **64**, 2487 (1931).



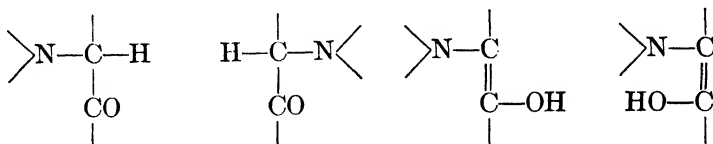
Extensive studies by Rabe⁸³ on the stereochemistry (p. 250) of the numerous isomers in the cinchona series have shown that, in the sixteen cinchona alcohols investigated, the steric arrangement of asymmetric atoms 3 and 4 is the same. Each of the pairs of isomers, cinchonine and cinchonidine



($R' = H$, $R = CH=CH_2$), hydrocinchonine and hydrocinchonidine ($R' = H$, $R = C_2H_5$), quinine and quinidine ($R' = OCH_3$, $R = CH=CH_2$), hydroquinine and hydroquinidine ($R' = OCH_3$, $R = C_2H_5$), yields one ketone on oxidation. This fact, however, does not

⁸³ Rabe, *Ber.*, **55**, 522 (1922); *Ann.*, **492**, 242 (1932).

supply valid proof that the isomerism depends upon the configuration at C-9 alone, since the ketones exist in solution as an equilibrium mixture of two keto and two enol forms.



Reduction of the ketone regenerates the two alkaloids together with two new epimeric alcohols. On conversion of hydrocinchoninone to the desoxy derivative (at C-9, $\text{CO} \rightarrow \text{CH}_2$), two stereoisomeric products are obtained, whence the conclusion can be drawn that both C-8 and C-9 are involved in the isomerism of the pairs named above. The two new alcohols obtained from reduction of a given cinchona ketone complete the number of stereoisomers (four) to be expected from configurational differences at the asymmetric centers C-8 and C-9.

Cupreine, $\text{C}_{19}\text{H}_{22}\text{O}_2\text{N}_2$, is found in the form of a molecular compound with quinine in the bark of *Remijia* varieties. It takes its name from the blue color of the bark. The structure of cupreine ($\text{R}' = \text{OH}$, $\text{R} = \text{CH}=\text{CH}_2$) is evident from the fact that it is phenolic in nature, and on methylation is converted to quinine. The ethyl ether of dihydrocupreine ($\text{R}' = \text{OC}_2\text{H}_5$, $\text{R} = \text{C}_2\text{H}_5$), a homolog of dihydroquinine, is an effective agent for the treatment of pneumococcus infections, and is used as the hydrochloride under the name "Optochin."

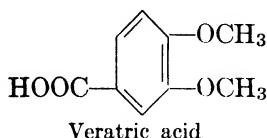
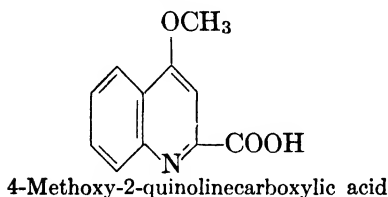
The cinchona alkaloids are marked by a toxic action on protoplasm, especially on low organisms, specifically on malaria parasites. Quinine is especially effective, and has in addition an antipyretic effect resulting from direct action on the heat-producing foci.

Angostura Alkaloids. The bark of *Galipea cusparia* (*Galipea officinalis*, angostura bark), which is employed in the West Indies as a febrifuge and finds further extensive use in bitter flavoring extracts, contains a variety of quinoline bases. The structures of some of these, cusparine, galipine, galipoline, and 2-*n*-amyl-4-methoxyquinoline, have been elucidated by E. Späth;⁸⁴ the nature of cuspareine and galipidine is still uncertain.

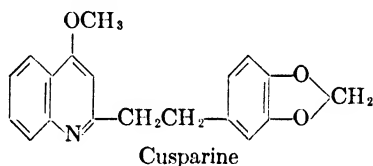
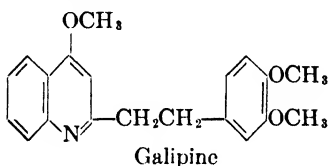
Galipine, $\text{C}_{20}\text{H}_{21}\text{O}_3\text{N}$, and cusparine, $\text{C}_{19}\text{H}_{17}\text{O}_3\text{N}$, both yield proto-catechuic acid when fused with alkali; this fact, together with the results of the methoxyl determination and the relationship of the empirical formulas, suggests that the second differs from the first only in containing

⁸⁴ Späth and co-workers, *Ber.*, **57**, 1243, 1687 (1924); *Monatsh.*, **52**, 129 (1929); **55**, 352 (1930).

a methylenedioxy group in place of two methoxyl groups. Controlled oxidation of galipine results in 4-methoxy-2-quinolinecarboxylic acid and veratric acid.



The formula derived for galipine by linkage of these two fragments through a C_2H_4 chain was shown to be correct by synthesis. 4-Methoxy-2-methylquinoline was condensed with veratraldehyde in the presence of zinc chloride, and the unsaturated product hydrogenated; the resulting base was identical with natural galipine. By a parallel reaction with piperonaldehyde, cusparine was obtained.



Galipoline is a phenolic base, differing from galipine in its formula by CH_2 . On methylation it is converted to galipine. A choice between the three possible formulas was made by synthesis, and galipoline was shown to be a galipine demethylated at the 4-position of the quinoline nucleus. According to Schöpf, these quinoline bases are probably formed in the plant through condensation of *o*-aminobenzaldehyde with various β -keto acids (p. 1109).

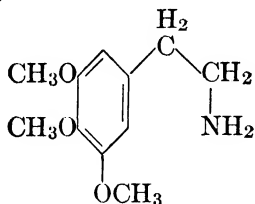
ISOQUINOLINE GROUP. MESCAL, HYDRASTIS, BERBERIS, AND OPIUM ALKALOIDS

Alkaloids containing the isoquinoline (or tetrahydroisoquinoline) nucleus are scattered through a number of plant families, the *Cactaceae*, *Papaveraceae*, *Ranunculaceae*, *Menispermaceae*, and others. Associated with them in a few cases are open-chain bases whose relationship to the cyclic alkaloids is close.

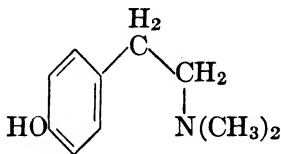
Mescal Alkaloids. The flowering heads of several varieties of *Anhalonium* or *Lophophora* cactus, known as mescal buttons, have long been used as an intoxicant ("pellote," "peyotl") by the natives of Mexico and the southwestern portion of the United States. Dried slices

of the plant are chewed as a part of primitive ceremonial rites, and aqueous or alcoholic extracts from the buttons are also consumed for their exhilarating effect. The excitement, and color and sound hallucinations experienced probably arise largely from the action of mescaline.

Mescaline is oxidized by potassium permanganate to trimethylgallic acid; its general behavior shows further that the basic portion of the molecule is not cyclic in nature. The structural formula rests on the syntheses of Späth⁸⁵ and others.⁸⁶ Anhaline likewise has its nitrogen atom in an open chain, and is identical with hordenine, a base found in barley germs.

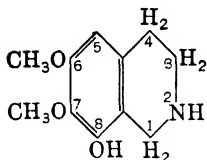


I. Mescaline

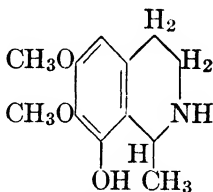


II. Anhaline (Hordenine)

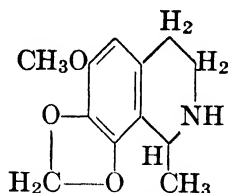
The remaining members of the anhalonium group, anhalamine, anhalonidine, anhalonine, pellotine, lophophorine, anhalinine, and anhalidine, are tetrahydroisoquinoline types.



III. Anhalamine



IV. Anhalonidine



V. Anhalonine

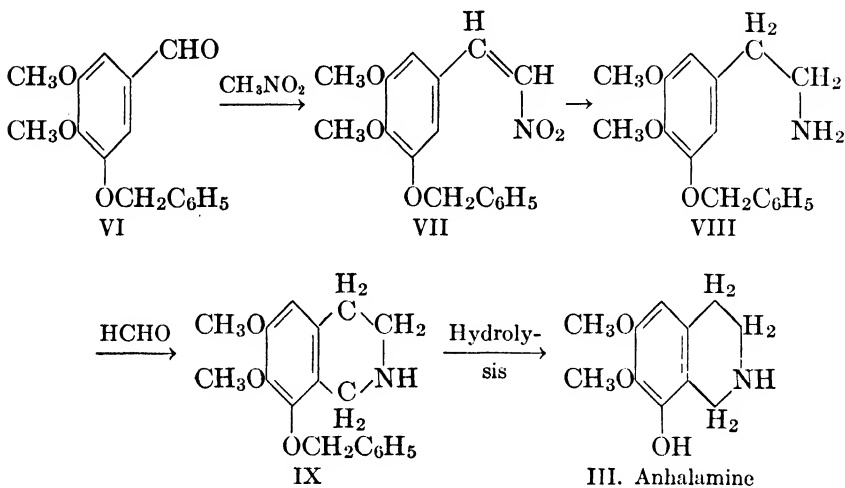
Pellotine and lophophorine are the N-methyl derivatives of anhalonidine and anhalonine respectively. Anhalinine represents the O-methyl derivative of anhalamine; anhalidine is the N-methyl derivative of anhalamine. The structure of the entire series has been demonstrated by synthesis.⁸⁷ The synthetic methods for the individual members of the group vary, but the synthesis of anhalamine may serve as an example. 3,4-Dimethoxy-5-benzyloxybenzaldehyde (VI) was condensed with nitromethane, and the ω -nitrostyrene derivative (VII) so obtained was

⁸⁵ Späth, *Monatsh.*, **40**, 129 (1919).

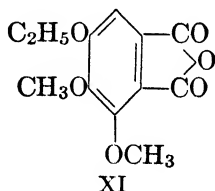
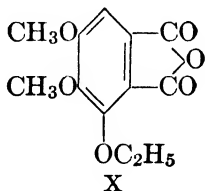
⁸⁶ Slotta and Heller, *Ber.*, **63**, 3029 (1930); Kindler and Peschke, *Arch. Pharm.*, **270**, 410 (1932); Hahn and Wassmuth, *Ber.*, **67**, 696 (1934).

⁸⁷ Späth and Becke, *Monatsh.*, **66**, 327 (1935).

reduced to the corresponding β -phenylethylamine derivative (VIII). Condensation of the phenylethylamine with formaldehyde resulted in closure of the tetrahydroisoquinoline ring (IX), and on removal of the benzyl group by hydrolysis anhalamine was obtained.

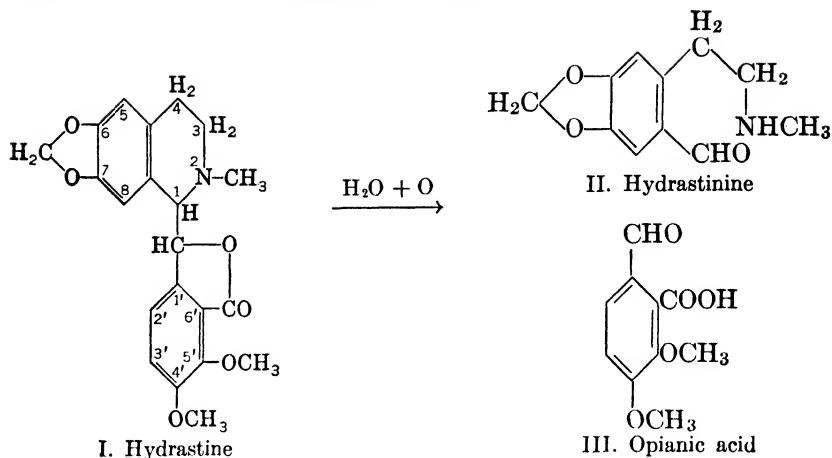


The tetrahydroisoquinoline ring closure, VIII to IX, might take place either *ortho* or *para* to the benzyloxy group. A decision in favor of the *ortho*-position was reached by degradation of anhalamine ethyl ether, which yielded the anhydride X, instead of XI, which must have resulted from the alternative possibility.

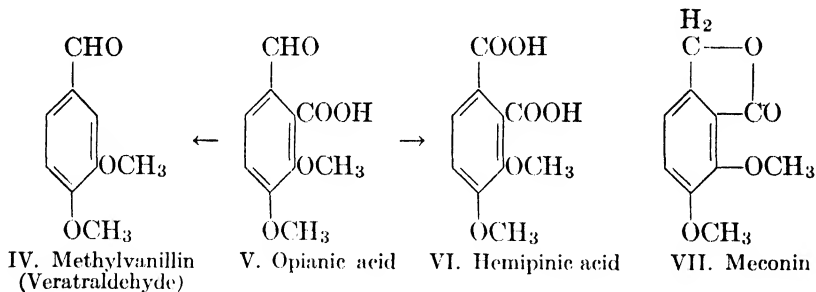


Hydrastis Alkaloids. The rhizomes of *Hydrastis canadensis* (golden seal) contain the three alkaloids hydrastine, berberine, and canadine, of which hydrastine, $\text{C}_{21}\text{H}_{21}\text{O}_6\text{N}$, is the most important. It is closely related to the opium alkaloid narcotine, which is 8-methoxyhydrastine; the two bases present a complete analogy in their reactions.

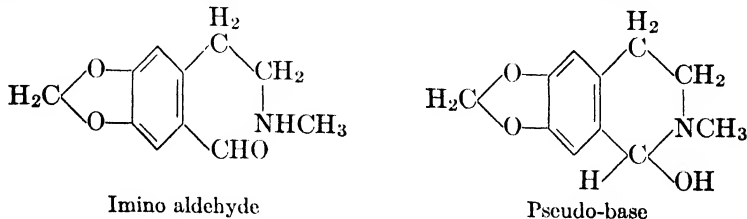
Hydrastine is a tertiary base carrying a methyl group on nitrogen. Two methoxyl groups and a methylenedioxy group are present; the remaining two oxygen atoms are found in a lactone linkage.



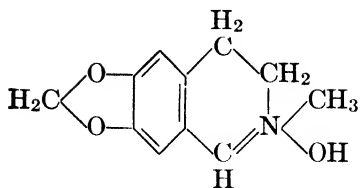
The hydrastine structural formula was developed largely through the researches of Freund and of E. Schmidt. On oxidative hydrolysis, the molecule is split into hydrastinine and opianic acid;⁸⁸ the structure of the latter is known from relationships to methylvanillin (decarboxylation) and to hemipinic acid (oxidation). On heating alone, hydrastine yields as the non-basic part meconin, the lactone of meconinic acid.



The basic portion from the above oxidative hydrolysis, hydrastinine, behaves as an aldehyde and as a secondary amine. Numerous reactions, as well as evidence from absorption spectra, indicate that hydrastinine (like its analog cotarnine) exists in three tautomeric forms:



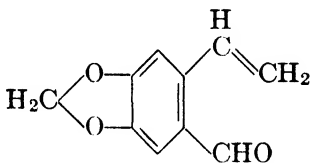
⁸⁸ Freund and Will, *Ber.*, **20**, 88 (1887).



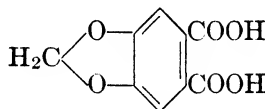
Quaternary base

Salt formation takes place through the quaternary ammonium form with loss of a molecule of water (Formula XII). Reduction of hydrastinine salts results in hydrohydrastinine (XV), an N-methyltetrahydroisoquinoline derivative.

Exhaustive methylation of hydrastinine gives the nitrogen-free aldehyde, hydrastal (VIII). The constitution of hydrastal rests upon oxidation to hydrastic acid (IX), the methylene ether of 4-5-dihydroxyphthalic acid. Hydrastic acid is known as the end product from the degradation of many natural substances, and has been synthesized in several ways.

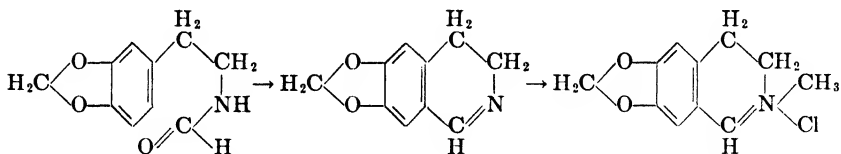


VIII. Hydrastal



IX. Hydrastic acid

The therapeutic value of hydrastinine in reducing uterine hemorrhage has led to the development of practicable syntheses⁸⁹ through modification of Decker's method.⁹⁰ In this synthesis, formylhomopiperonylamine is subjected to a Bischler-Napieralski reaction,⁹¹ a cyclodehydration accomplished in the presence of phosphorus pentachloride, and the resulting norhydrastinine is converted to hydrastinine salts by the action of methyl halides.



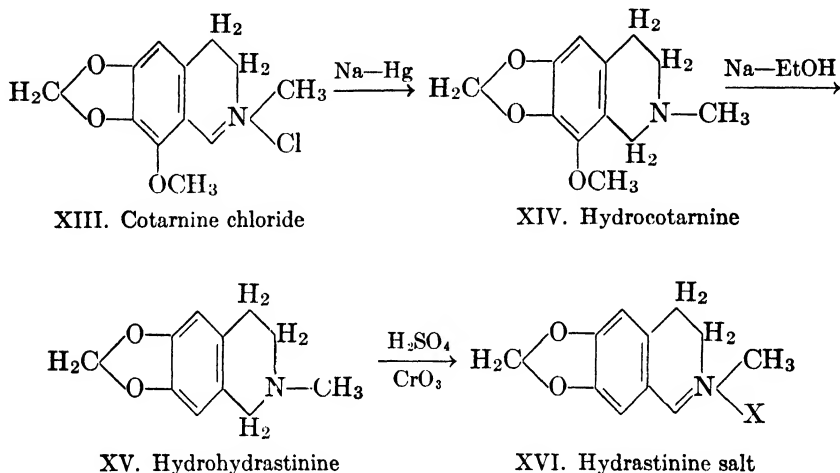
X. Formylhomopiperonylamine XI Norhydrastinine XII. Hydrastinine chloride

⁸⁹ Rosenmund, *Ber. deut. pharm. Ges.*, **29**, 200 (1919); Kindler and co-workers, *Ann.*, **431**, 228 (1923); *Arch. Pharm.*, **265**, 389 (1927); **270**, 353 (1932).

⁹⁰ Decker and co-workers, *Ann.*, **395**, 299, 321, 328 (1913).

⁹¹ Bischler and Napieralski, *Ber.*, **26**, 1903 (1893).

Hydrastinine can also be prepared from the less valuable cotarnine (8-methoxyhydrastinine), a degradation product of narcotine. Cotarnine is reduced in acid medium to hydrocotarnine (XIV) (p. 1075), and the latter, on further reduction with sodium and alcohol, suffers replacement of the methoxyl group by hydrogen.⁹² The resulting hydrohydrastinine (XV) is converted by oxidation into hydrastinine.



Linkage of the meconin and hydrastinine nuclei as in formula I is assumed in analogy with the structure demonstrated for narcotine (p. 1074). Attempts to synthesize *d,l*-hydrastine by a method successfully employed in the preparation of *d,l*-narcotine have resulted in two inactive hydrastine isomers, whose relation to natural *l*-hydrastine is not known.⁹³

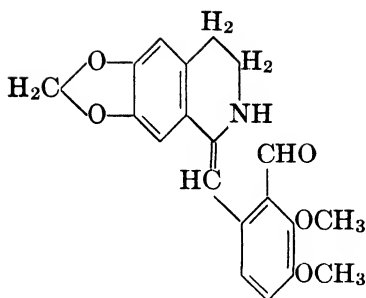
The alkaloid berberine, C₂₀H₁₉O₅N, is found not only in *Hydrastis*, but also in a number of unrelated plant families, notably the *Berberidaceae*, from which it takes its name. The structural formula has been developed largely through the researches of W. H. Perkin, jun.⁹⁴

Berberine, like hydrastinine and cotarnine, forms its salts with loss of a molecule of water, and like these bases, behaves as though it existed in three forms:

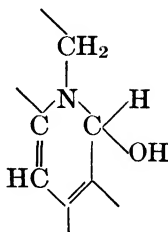
⁹² Pyman and Remfry, *J. Chem. Soc.*, **101**, 1595 (1912). This unusual type of reaction has been observed with a number of derivatives of pyrogallol trimethyl ether.

⁹³ Hope, Pyman, Remfry, and Robinson, *J. Chem. Soc.*, 236 (1931).

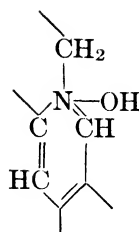
⁹⁴ Perkin, jun., *ibid.*, **55**, 63 (1889); **57**, 992 (1890); Perkin and Robinson, *ibid.*, **97**, 305 (1910); Perkin, *ibid.*, **113**, 492, 722 (1918).



XVII. Imino aldehyde

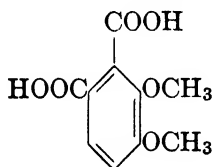


XVIIa. Pseudo-base

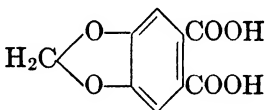


XVIIb. Quaternary base

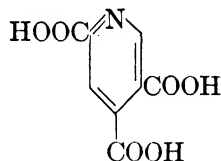
Information concerning the structure of berberine has been obtained largely through oxidation. With permanganate, hemipinic acid, hydrastinic acid, oxyberberine, and berberal are obtained; with nitric acid, berberonic acid results.



VI. Hemipinic acid



IX. Hydrastinic acid



XVIII. Berberonic acid

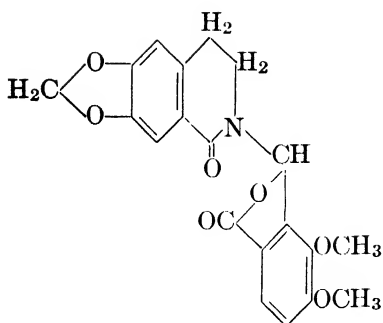
The construction of a reasonable berberine formula from these smaller fragments was accomplished through a study of berberal. This base breaks down in hydrolytic processes into noroxyhydrastinine and pseudoopianic acid; these two components can also be united with loss of water to give berberal. Pseudoopianic acid is an isomer of the opianic and metaopianic acids arising from the degradation of narcotine and cryptopine, respectively; in combining with noroxyhydrastinine it is assumed to react in the hydroxyphthalide form (XXI), and the product, berberal, is given the structure XIX. The structure of noroxyhydrastinine was shown by the relationship to the N-methyl derivative, oxyhydrastinine, a substance obtained from hydrastinine by Cannizzaro's reaction.

From a consideration of berberal and of the reactions of berberine, Perkin, Gadamer,⁹⁵ and Faltis⁹⁶ advanced the now accepted formula of berberine, which has been substantiated by several syntheses.⁹⁷ The

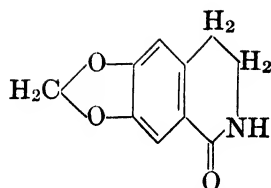
⁹⁵ Gadamer, *Arch. Pharm.*, **239**, 648 (1901).

⁹⁶ Faltis, *Monatsh.*, **31**, 557 (1910).

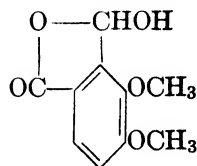
⁹⁷ Pictet and Gams, *Compt. rend.*, **152**, 1102 (1911); **153**, 386 (1911); *Ber.*, **44**, 2036, 2480 (1911); Perkin, *J. Chem. Soc.*, **113**, 737 (1918); Haworth, Perkin, and Rankin, *ibid.*, **125**, 1686 (1924); Perkin, Rây, and Robinson, *ibid.*, **127**, 740 (1925); Späth and Quietensky, *Ber.*, **58**, 2267 (1925).



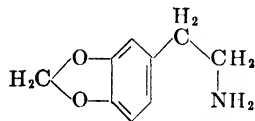
XIX. Berberal



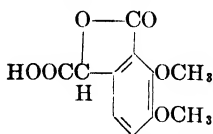
XX. Noroxyhydrastinine

XXI. Pseudoopianic acid
(Hydroxyphthalide form)

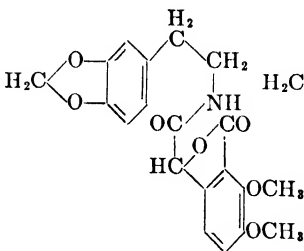
synthetic methods have in general as their goal oxyberberine (XXV), a base that has been obtained by gentle oxidation of berberine, or that is formed along with hydroberberine by a Cannizzaro intermolecular oxidation-reduction reaction when berberine is heated with alkali. In this change, berberine must be considered as reacting in the pseudo-base form XVIIa. Oxyberberine can be reduced to tetrahydroberberine, and the latter converted to berberine by oxidation. The oxyberberine synthesis of Perkin, Rây, and Robinson consisted in condensation of homopiperonylamine and meconincarboxylic acid to an amide (XXIV), which was then subjected to a Bischler-Napieralski isoquinoline ring closure. This resulted in an intermediate, which was reducible to oxyberberine.



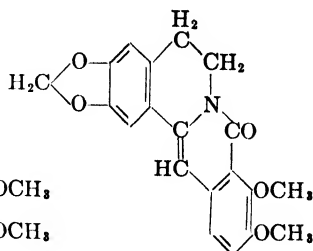
XXII. Homopiperonylamine



XXIII. Meconincarboxylic acid



XXIV. Meconincarboxyhomopiperonylamide



XXV. Oxyberberine

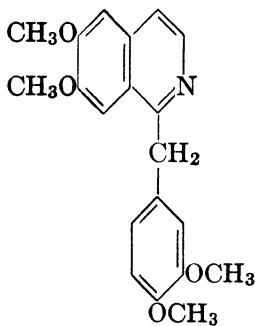
Berberine is relatively inactive physiologically; in large doses it exerts a paralyzing effect of central origin. It has been variously recommended as an oxytocic, as an antimalarial, and as a cure for morphinism.

Canadine is *l*-tetrahydroberberine,^{95,98} and occurs also as the dextro form in *Corydalis*⁹⁹ along with other alkaloids (corydaline, corybulbine, etc.) closely related to berberine.

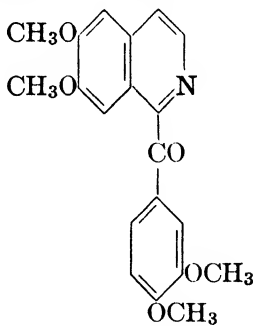
Opium Alkaloids.¹⁰⁰ Opium is the dried latex from the unripe seed capsules of the opium poppy, *Papaver somniferum*, and has proved to be one of the richest sources of alkaloids. The bases occur in part in the free state, in part combined with sulfuric, lactic, acetic, or meconic acids. Most of them are derivatives of isoquinoline or tetrahydroisoquinoline; the remainder (morphine group) are probably related phytochemically to those of the isoquinoline group,^{1,2,3} and are most conveniently considered under this classification.

Because of the great importance of opium and its alkaloids in medicine, the world production is enormous. In 1933, 1,913,834 kg. of raw opium were reported to the League of Nations, and in the same year the manufacture of 30,788 kg. of morphine alkaloid was recorded. These figures, however, do not include the huge quantities produced illegitimately to supply the needs of opium and morphine addicts. The clandestine production probably exceeds 5000 tons of opium.

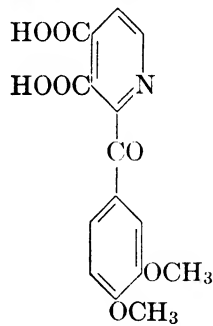
Papaverine, $C_{20}H_{21}O_4N$, is found in all parts of the growing poppy, and is present in opium to the extent of about 0.5 to 1 per cent. Its structure as tetramethoxy-1-benzylisoquinoline was elucidated in a long series of researches by Goldschmiedt.¹⁰¹ These investigations constitute an excellent example of the application of oxidative degradation to structure determination. By gentle oxidation of papaverine, the secondary alcohol papaverinol is formed;¹⁰² more vigorous treatment



I. Papaverine



II. Papaveraldine



III. Papaverinic acid

⁹⁸ Gadamer, *Arch. Pharm.*, **248**, 43 (1910).

⁹⁹ Späth and Julian, *Ber.*, **64**, 1131 (1931).

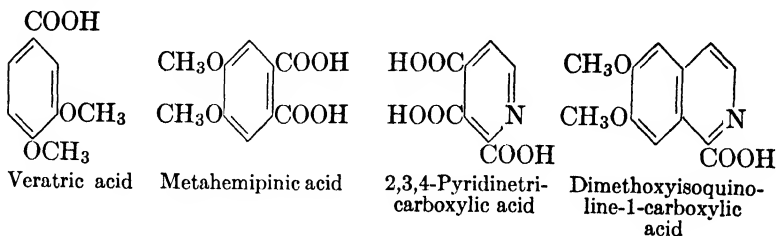
¹⁰⁰ Small and Lutz, "Chemistry of the Opium Alkaloids," U. S. Government Printing Office (1932); Kappelmeier, "Die Konstitutionserforschung der wichtigsten Opium Alkaloide," Ahrens' "Samml. chem. chem.-tech. Vorträge," Vol. 18, p. 225 (1912).

¹⁰¹ Goldschmiedt, *Monatsh.*, **9**, 778 (1888).

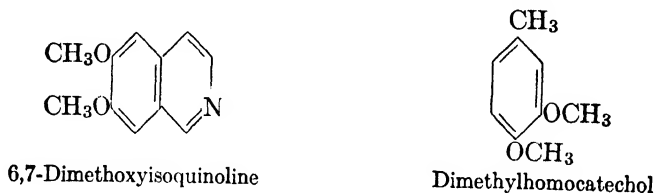
¹⁰² Gadamer and Schulemann, *Arch. Pharm.*, **253**, 284 (1915).

yields the corresponding ketone, papaveraldine,¹⁰³ or finally the dibasic acid, papaverinic acid.¹⁰⁴

On more complete oxidation, the fragments obtained are veratric acid, metahemipinic acid, 2,3,4-pyridinetricarboxylic acid, and 6,7-dimethoxyisoquinoline-1-carboxylic acid.¹⁰⁴



On fusion with potassium hydroxide, papaverine yields among other products, 6,7-dimethoxyisoquinoline and dimethylhomocatechol.¹⁰⁵



The mode of union of these fragments in papaverine is evident: the appearance of two methoxyl groups in each portion, as well as in the oxidation products above, shows that the methoxyls do not take part in the linkage; a direct union of two aromatic nuclei would not explain the ease with which they separate. Linkage through a methylene group at the point where the carboxyl of dimethoxyisoquinoline-1-carboxylic acid is found gives a satisfactory explanation of these facts and of the other reactions of papaverine.

Papaverine was first synthesized by Pictet and Gams¹⁰⁶ by a method that gave complete confirmation to the accepted structure. As starting substances for this synthesis, veratrole (*o*-dimethoxybenzene) and veratric acid (3,4-dimethoxybenzoic acid) were chosen. By the Friedel and Crafts reaction veratrole was converted to acetoveratrone, and the isonitroso derivative of acetoveratrone was reduced with tin and hydrochloric acid to aminoacetoveratrone (IV). Interaction of aminoacetoveratrone hydrochloride and homoveratroyl chloride (V) yielded the

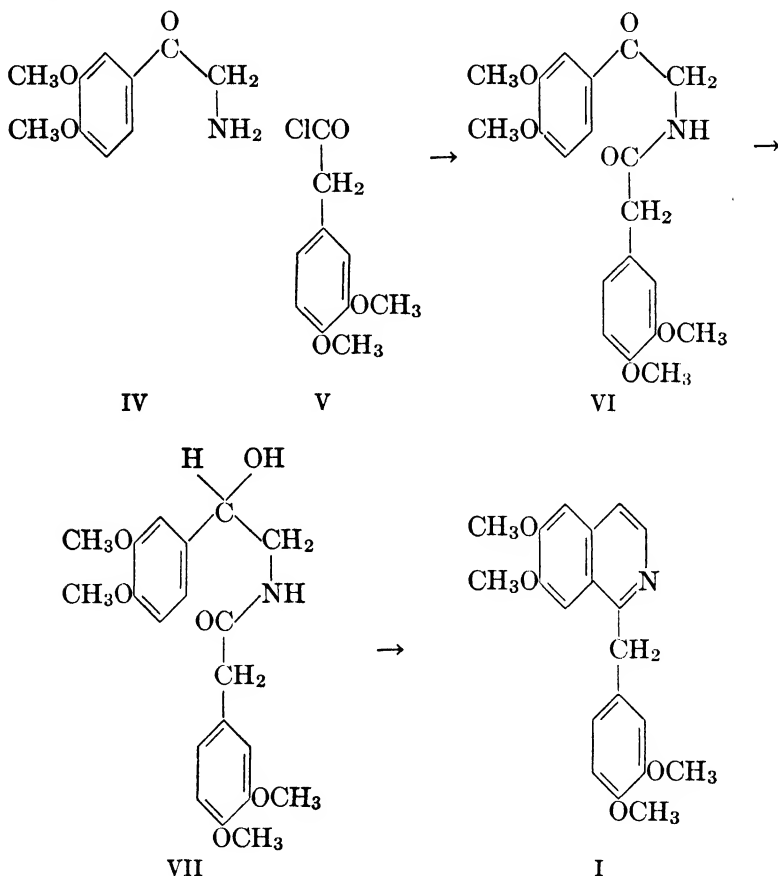
¹⁰³ Goldschmiedt, *Monatsh.*, **6**, 954 (1885); **7**, 485 (1886).

¹⁰⁴ Goldschmiedt, *ibid.*, **6**, 372 (1885); Goldschmiedt and Strache, *ibid.*, **10**, 692 (1889).

¹⁰⁵ Goldschmiedt, *ibid.*, **8**, 510 (1887).

¹⁰⁶ Pictet and Gams, *Ber.*, **42**, 2943 (1909).

amide, ω -(homoveratroylamido)acetovertatrone (VI). By selective reduction of the ketonic carbonyl group of VI with sodium amalgam, the corresponding secondary alcohol, homoveratroylhydroxyhomoveratroylamine, was obtained. This substance, heated in xylene with phosphorus pentoxide, lost two molecules of water, closing the isoquinoline ring to give papaverine.



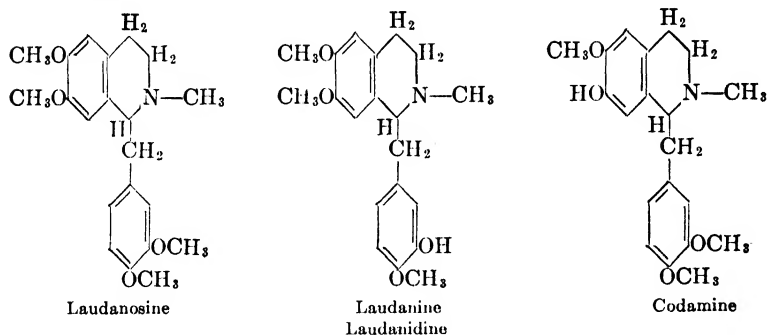
Numerous other syntheses have been developed;¹⁰⁷ it is reported that secret processes developed by drug manufacturers permit the synthesis of papaverine on any desired scale.

Papaverine causes light narcosis, in larger doses tetanus and res-

¹⁰⁷ Buck, Haworth, and Perkin, jun., *J. Chem. Soc.*, **125**, 2176 (1924); Rosenmund, Nothnagel, and Riesenfeldt, *Ber.*, **60**, 392 (1927); Späth and Burger, *Ber.*, **60**, 704 (1927); Buck, *J. Am. Chem. Soc.*, **52**, 3610 (1930); Späth and Berger, *Ber.*, **63**, 2098 (1930); Mannich and Walther, *Arch. Pharm.*, **265**, 1 (1927).

piratory paralysis. It has an antispasmodic action on smooth muscle and is used (chiefly in Europe) to relieve bronchial or intestinal spasms, and in obstetrics.

Laudanosine, $C_{21}H_{27}O_4N$, is found in opium in small amounts, and is closely related to papaverine. Its structure as *dextro*-tetrahydro-N-methylpapaverine was demonstrated by reduction of papaverine methochloride with tin and acid, and resolution of the resulting *d,l*-tetrahydro-N-methylpapaverine (racemic laudanosine); the *dextro* form was identical with laudanosine.¹⁰⁸ The first complete synthesis of laudanosine was carried out by Pictet and Finkelstein¹⁰⁹ and is of interest as the first synthesis of an opium alkaloid. In connection with laudanosine, the rare opium alkaloids laudanine, laudanidine, and codamine may be mentioned. Laudanine is the racemic form of 3'-demethylo-tetrahydro-N-methylpapaverine;¹¹⁰ laudanidine is the *levo* form of the same base.¹¹¹ Codamine represents racemic 7-demethylo-tetrahydro-N-methylpapaverine.¹¹²



The location of the phenolic hydroxyl groups in laudanine and codamine was shown by Späth through the device of ethylation and subsequent oxidation. From ethyllaudanine, 3-ethoxy-4-methoxybenzoic acid was obtained, from ethylcodamine an isoquinoline derivative carrying the ethoxyl group in position-7; the structure of both alkaloids was then confirmed by synthesis.

The alkaloid narcotine, $C_{22}H_{23}O_7N$, occurs in opium as the free base in amounts up to 10 per cent or more. It differs structurally from hydrastrine (p. 1066) only by the presence of a methoxyl group in the 8-position.

¹⁰⁸ Pictet and Athanasescu, *Ber.*, **33**, 2346 (1900).

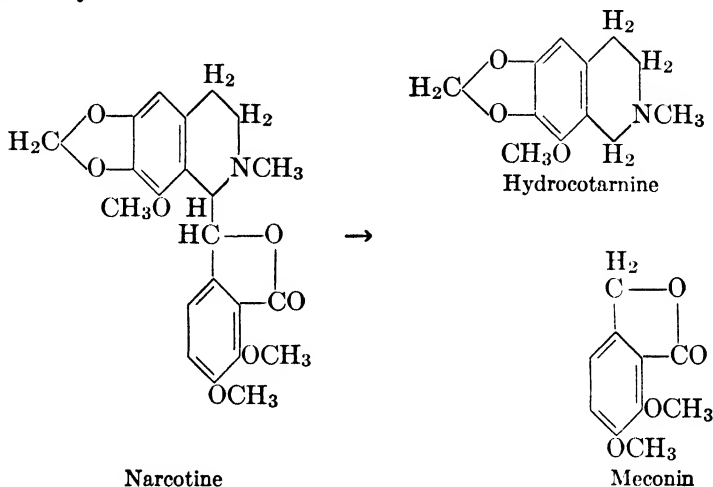
¹⁰⁹ Pictet and Finkelstein, *Ber.*, **42**, 1979 (1909).

¹¹⁰ Späth and Lang, *Monatsh.*, **42**, 273 (1921).

¹¹¹ Späth and Bernhauer, *Ber.*, **58**, 200 (1925); Späth and Burger, *Monatsh.*, **47**, 733 (1926).

¹¹² Späth and Epstein, *Ber.*, **59**, 2791 (1926); **61**, 334 (1928).

On oxidative hydrolysis it is broken down to cotarnine (the methoxy analog of hydrastinine) and opianic acid.^{113,114} From reductive hydrolysis, the fragments are meconin and the previously known opium alkaloid hydrocotarnine.¹¹⁴



Cotarnine presents in its reactions and tautomeric behavior a complete analogy to hydrastinine. On reduction it yields hydrocotarnine, the analog of hydrohydrastinine; on oxidation the product is cotarnic acid (methoxyhydrastic acid). When treated with bromine, cotarnine is converted to a series of hydroxyisoquinoline betaines known as tarconines.¹⁰⁰

The structural formula of narcotine, like that of hydrastine, was evolved by joining in the most reasonable manner the products identified from degradation. The presence of a tertiary nitrogen atom shows that the nitrogen-containing portion in narcotine has the isoquinoline structure of hydrocotarnine and not the open-chain amine (or tautomeric) form of cotarnine. The lactone nature of narcotine indicates the meconin, rather than the opianic acid, grouping for the nitrogen-free portion, and the appearance of two aldehyde groups (in cotarnine and opianic acid) in oxidative degradation shows the points at which the two fragments are joined.¹¹⁵ The structural formula so reached was confirmed by the synthesis of Perkin and Robinson.¹¹⁶ Meconin and

¹¹³ Wöhler, *Ann.*, **50**, 1 (1844).

¹¹⁴ Beckett and Wright, *J. Chem. Soc.*, **23**, 573 (1875); Rabe and McMillan, *Ber.*, **43**, 800 (1910).

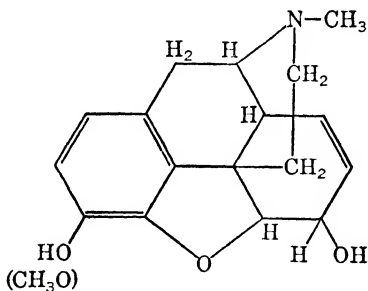
¹¹⁵ Roser, *Ann.*, **254**, 356 (1889).

¹¹⁶ Perkin and Robinson, *J. Chem. Soc.*, **99**, 775 (1911).

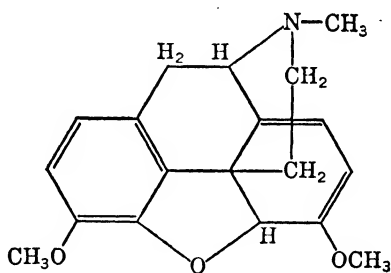
cotarnine (the latter probably reacting as the pseudo-base) were condensed, giving the opium alkaloid gnoscopine, which is *d,l*-narcotine; the *levo* form is natural narcotine. Since both of the constituents have been synthesized, this constitutes a complete narcotine synthesis.

The Morphine Alkaloids. Morphine was the first organic base to be isolated and characterized as such (Sertürner, 1805);¹¹⁷ it is today one of the most useful drugs known. Opium may contain as much as 20 per cent morphine, but the average is in the neighborhood of 10 per cent. Smoking opium, a specially prepared form, has about 8 per cent morphine.¹¹⁸ The methyl ether of morphine, known as codeine, and the third member of the morphine group, thebaine, are present to the extent of about 0.5 per cent in opium. It is not known with certainty whether morphine and codeine occur in any plant but *Papaver somniferum*; thebaine has been found in *Papaver orientale*.

No group of alkaloids has offered more stubborn resistance to solution of the structural problem; since 1889, when the first well-founded speculation appeared (Knorr¹¹⁹), no less than twenty structural formulas for morphine have been proposed by eminent workers in the field.¹⁰⁰ The most probable of these, advanced by Gulland and Robinson in 1925,^{1,120} is based upon the enormous amount of experimental evidence that has been accumulated in the last four decades, and explains best the complicated and exceptional reactions of the morphine group.



I. Morphine (Codeine)



II. Thebaine

Of the three oxygen atoms in morphine, $C_{17}H_{19}O_3N$, one is present in a phenolic hydroxyl, one in an alcoholic hydroxyl, and the third is indifferent, in an ether linkage. The nature of the last-named was

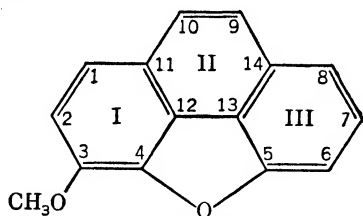
¹¹⁷ Krömeke, "Fr. Wilh. Sertürner, der Entdecker des Morphiums," Fischer, Jena (1925).

¹¹⁸ Sirans, *J. Ind. Eng. Chem.*, **8**, 345 (1916).

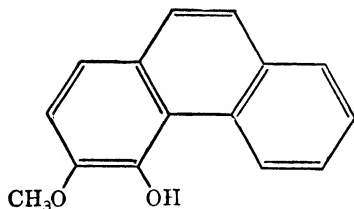
¹¹⁹ Knorr, *Ber.*, **22**, 1113 (1889).

¹²⁰ Gulland and Robinson, *J. Chem. Soc.*, **123**, 980 (1923).

shown by Vongerichten¹²¹ through his studies on methylmorphenol. This substance is formed in the last step of the exhaustive methylation of morphine (or codeine) through a reaction peculiar to the morphine series.



III. Methylmorphenol



IV. Methylmorphol

Methylmorphenol is 3-methoxy-4,5-phenanthrylene oxide; it can be transformed to 3-methoxy-4-hydroxyphenanthrene (methylmorphol) by reduction with sodium and alcohol, or to 3,4,5-trihydroxyphenanthrene by alkali fusion. The location of two of the morphine oxygen atoms and the presence of the phenanthrene nucleus are thus demonstrated. By zinc dust distillation of morphine and its derivatives, phenanthrene itself is obtained.

Both hydroxyl groups of morphine are acetylated by acetic anhydride, yielding diacetylmorphine, whose hydrochloride is the important narcotic, Heroin. With chlorinating agents only the alcoholic hydroxyl group is attacked, and a phenolic halogenated base, α -chloromorphide, results; at the same time small amounts of an isomer, β -chloromorphide, are formed. The β -isomer represents a rearrangement product of the α -compound, and can be prepared from it; the nature of the isomerism is not certain, but it is probably due to attachment of the halogen at a different point in ring III.

The two chloromorphides can be hydrolyzed to three isomers, known as α -, β -, and γ -isomorphine.¹²² No morphine is regenerated in the hydrolysis. In nearly all morphine studies, structural determination has been made in the methyl ether (codeine) series because of the greater stability and more agreeable physical properties of these derivatives. In the reactions under consideration, codeine, through α - or β -chlorocodide, is converted to isocodeine, allopseudocodeine, and pseudocodeine, corresponding respectively to α -, β -, and γ -isomorphines.

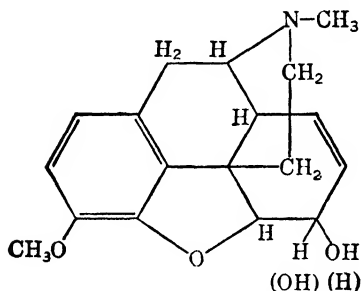
Knorr¹²³ was able to show that codeine and isocodeine can be oxidized at the alcoholic hydroxyl group to give the same ketone,

¹²¹ Vongerichten, *Ber.*, **30**, 2439 (1897); **31**, 3198 (1898); **33**, 352 (1900).

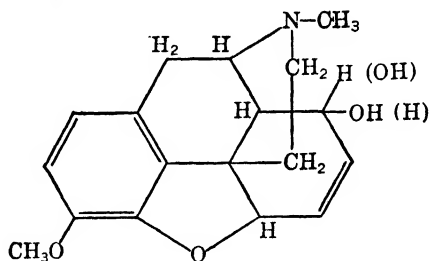
¹²² Lees, *J. Chem. Soc.*, **91**, 1408 (1907); Oppé, *Ber.*, **41**, 975 (1908).

¹²³ Knorr and Hörlein, *Ber.*, **40**, 2032, 3341, 4889 (1907); Knorr, *Ber.*, **36**, 3074 (1903).

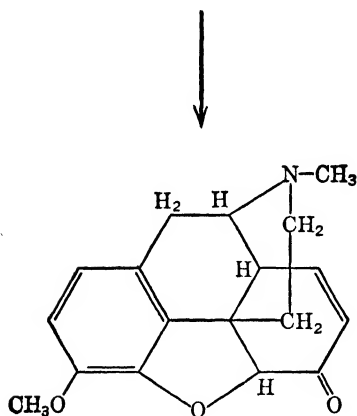
codeinone; the isomerism depends therefore only upon the spatial arrangement of hydrogen and hydroxyl in these two diastereoisomers. Codeinone, moreover, can be degraded to 3,4,6-trimethoxyphenanthrene. The alcoholic hydroxyl group in codeine and isocodeine, as well as in morphine and α -isomorphine, must be located on carbon -6. By a



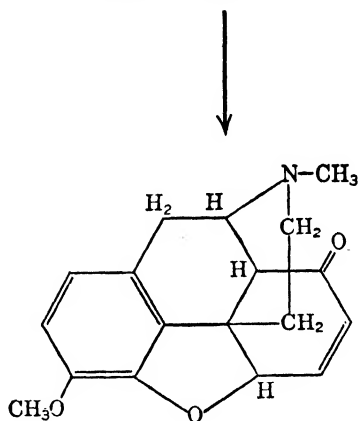
V. Codeine, Isocodeine



VI. Allopseudocodeine, Pseudocodeine



VII. Codeinone



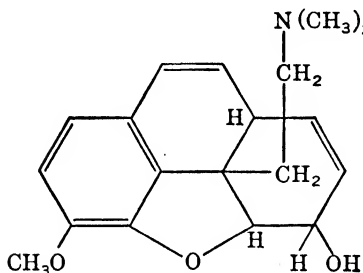
VIII. Pseudocodeinone

similar method, through pseudocodeinone and 3,4,8-trimethoxyphenanthrene it was shown that in allopseudo- and pseudo-codeines the hydroxyl group is on carbon-8. The nuclear positions -6 and -8 are thus excluded as possible points of attachment of the nitrogen-containing ring.

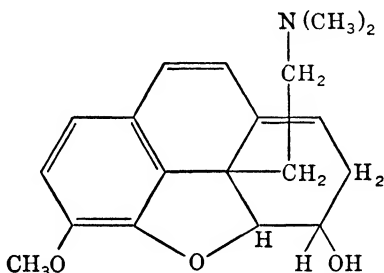
The presence of the alicyclic double bond in morphine and codeine and in the isomers can be demonstrated by catalytic hydrogenation; in pseudocodeine and allopseudocodeine a tendency to add four atoms of hydrogen with reductive scission of the 4,5-ether linkage is seen, a phenomenon that is undoubtedly connected with the allyl ether struc-

ture of these bases. The position assigned to the codeine double bond depends upon reactions of the methylmorphimethines.

Satisfactory proof of the position of the nitrogen-containing ring in the morphine series has been most difficult to obtain. The nitrogen atom is tertiary and carries a methyl group. When codeine methiodide is heated with alkali, the nitrogen ring is broken in the usual way and the product is α -methylmorphimethine.¹²⁴



IX. α -Methylmorphimethine



X. β -Methylmorphimethine.

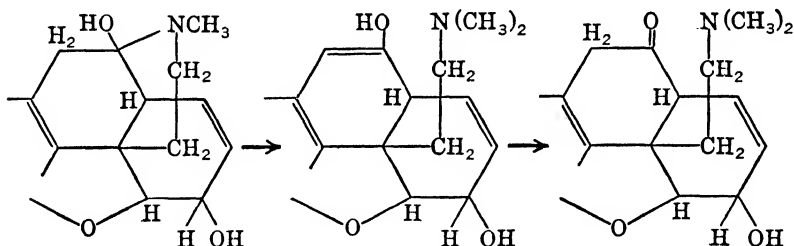
Under the influence of alcoholic alkali, α -methylmorphimethine is transformed to an isomer, β -methylmorphimethine. The change is believed to be due to a shift of the 7,8-double bond to a position (-8,14) in conjugation with the new unsaturation at -9,10, and is one of the chief reasons for placing the morphine double linkage at position -7,8. These two methine bases yield the same tetrahydro derivative, showing that the isomerism is due only to a difference in position of the double bond. By the same degradation process, isocodeine gives γ -methylmorphimethine, which likewise can undergo rearrangement. From pseudocodeine and allopseudocodeine, however, methylmorphimethines (ϵ - and ζ -) are obtained in which the location of the unsaturation and hydroxyl is such as to preclude a shift to form a conjugated system.^{120,125}

When the methylmorphimethines are heated with acetic anhydride, they break down into methylmorphol (IV) and β -hydroxyethyltrimethylamine, $(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OH}$. The nitrogen atom in the methines and in morphine is evidently linked with two carbon atoms in a chain which is easily separated as a whole from the phenanthrene nucleus. Degradation of the methylmorphimethine methiodides with alkali by the usual Hofmann procedure also results in loss of the chain in the form of trimethylamine and ethylene. The point of attachment of the nitrogen

¹²⁴ Hesse, *Ann.*, **222**, 203 (1883).

¹²⁵ Knorr and co-workers, *Ber.*, **35**, 3009, 3010 (1902); **39**, 4412 (1906); **40**, 3844 (1907); Schryver and Lees, *J. Chem. Soc.*, **79**, 563 (1901); Wieland and Koralek, *Ann.*, **433**, 267 (1923).

atom to the nucleus in morphine rests on Knorr's¹²⁶ studies of 9-hydroxycodeine, a derivative obtained by gentle oxidation of codeine. The methylmorphimethine formed in the first step of the degradation of this hydroxycodeine is a ketone, hence the new hydroxyl group must be located on a carbon atom that becomes unsaturated when the nitrogen-containing ring opens.



Only positions -9 or -10 are possible for the carbon atom in question, for acetolysis of the methine results in a methoxydiacetoxypheanthrene that cannot be oxidized to a phenanthrene-9,10-quinone without loss of an acetoxy group.

Because of the great lability of the ethanamine chain, the location of its other end has been fraught with much difficulty. Treatment of morphine with various acidic reagents results in apomorphine, in which Pschorr demonstrated, both by degradation and by synthesis, that the chain is linked at position-8.¹²⁷ This position is untenable for morphine, however, because of the evidence cited above for the structure of pseudocodeinone. Thebaine, which is known through its relationship to codeinone and to dihydromorphine dimethyl ether to contain the fundamental morphine skeleton, may give through the action of hydrochloric acid either morphothebaine (chain on carbon-8) or thebenine, in which the chain is unquestionably attached at carbon-5.¹²⁸

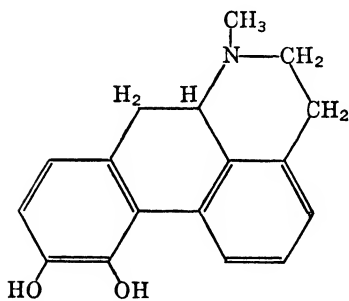
The generally accepted location of the chain at carbon-13 was developed in an attempt to account for the extraordinary tendency shown by all the members of the morphine group to lose the entire ethanamine side chain in degradative reactions. Linkage at a quaternary carbon atom (position-13 or -14) is the only arrangement under which it becomes necessary for the side chain to shift (to position-5 or -8) or separate from the molecule when aromatization of ring III or of the whole phenanthrene nucleus takes place. Position-14 is improbable because it does not

¹²⁶ Knorr and co-workers, *Ber.*, **39**, 1414 (1906); **40**, 2042 (1907).

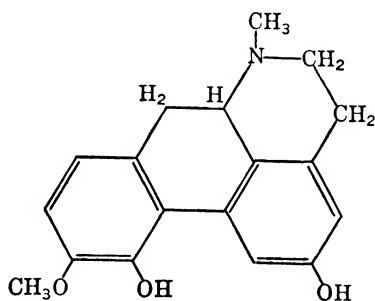
¹²⁷ Pschorr and co-workers, *Ber.*, **40**, 1998 (1907); **62**, 321 (1929); Späth and Hromatka, *Ber.*, **62**, 325 (1929).

¹²⁸ Gulland and Virden, *J. Chem. Soc.*, 921 (1928).

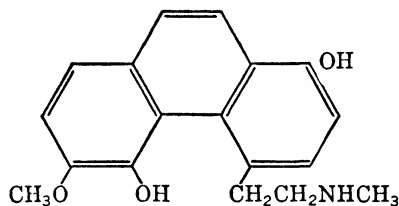
permit of a reasonable structure for thebaine. Schöpf² was able to substantiate the structural theory of Gulland and Robinson by a study



XI. Apomorphine.

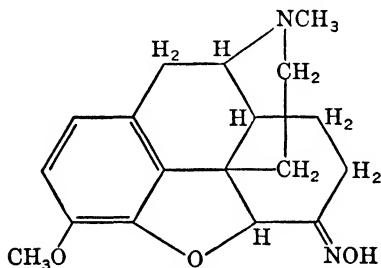


XII. Morphothebaine.

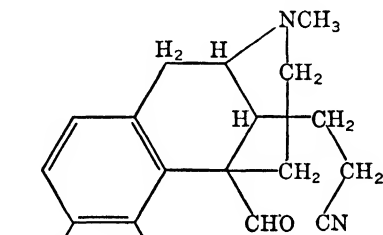


XIII. Thebaine.

of the Beckmann rearrangement of dihydrocodeinone oxime (XIV), which resulted in formation of an aldehyde (XV), instead of the ketone that would be expected if the chain were attached in position-5.



XIV



XV

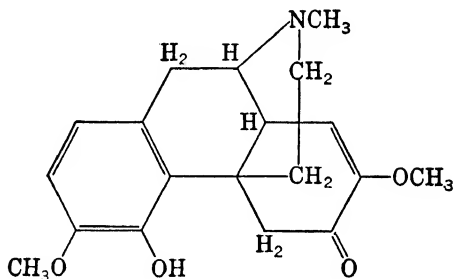
Thebaine (II) is regarded as the methyl ether of the enol form of codeinone (VII), and can be converted to codeinone by gentle hydrolysis. When the two hydroaromatic double bonds present in thebaine are saturated, dihydromorphine dimethyl ether is obtained.

In recent years, exhaustive researches have been carried out to determine the structural features responsible for the physiological action of morphine. It may be stated briefly that the presence of the phenolic

hydroxyl is essential for high analgesic action, while the alcoholic hydroxyl appears to exert an opposite effect. When the alcoholic hydroxyl group is replaced by hydrogen, or methylated, a great increase in analgesic power is observed. Morphine alcoholic methyl ether, for example, is approximately one hundred times as effective in this respect as the phenolic methyl ether (codeine). If the nitrogen- or oxygen-containing rings of morphine are opened, a great decrease in physiological action results.

Neopine, a recently discovered rare member of the morphine group, represents a codeine in which the alicyclic unsaturation lies between carbons-8 and -14. It is converted to dihydrocodeine on hydrogenation, or directly to β -methyilmorphimethine in the first step of Hofmann's degradation.¹²⁹

An alkaloid having a structural skeleton similar to that of the morphine group is found in the Japanese vine *Sinomenium acutum*.¹³⁰ This base, sinomenine, is a 7-methoxy derivative of the keto phenol thebainone in the morphine series.



All the asymmetric carbon atoms in sinomenine have the configuration opposite to that of the corresponding asymmetric centers in morphine; conversion of sinomenine to the optical antipodes of morphine derivatives has been accomplished.

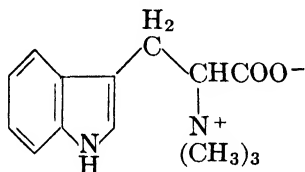
Morphine finds therapeutic use as a result of its depressant action on different parts of the central nervous system. It causes marked analgesia and, in larger doses, narcosis. Codeine has more tendency to excite, and the narcotic effects of morphine are exhibited, but in weaker degree. Morphine and many of its derivatives are characterized by their ability to produce the dangerous addiction known as morphinism. Thebaine is a violent tetanic poison.

¹²⁹ Van Duin, Robinson, and Smith, *ibid.*, 903 (1926).

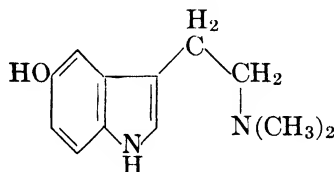
¹³⁰ Kondo and Ochiai, *Ann.*, **470**, 224 (1929).

**INDOLE GROUP. HYPAPHORINE, ABRINE, AND GRAMINE;
HARMALA, PHYSOSTIGMINE, YOHIMBINE, STRYCHNOS, AND
ERGOT ALKALOIDS**

The important group of alkaloids containing the indole nucleus ranges in complexity from such simple substances as hypaphorine (I), the methylbetaine of tryptophan, to the complicated structures of yohimbine and strychnine. Probably all these alkaloids have as the parent substance the amino acid tryptophan (p. 940), a building unit that appears to be of great importance in the synthesis of both plant and animal bases. The toad poison (p. 947) bufotenine is a derivative of tryptamine; it is interesting to note that bufotenine and physostigmine (p. 1086) are the only 5-hydroxyindole derivatives that have been encountered in nature.¹³¹

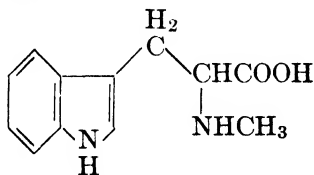


I. Hypaphorine

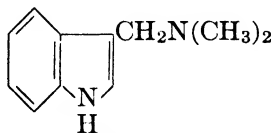


II. Bufotenine

Abrine, an alkaloid from the seeds of *Abrus precatorius*, is likewise a simple derivative of tryptophan. Its constitution as in formula III is immediately evident from the fact that it can be decarboxylated to yield N-methyltryptamine. The optical activity of abrine excludes a ring position for the carboxyl group. On treatment with methyl iodide and alkali, moreover, it gives the same methyl ester methiodide as is obtained from the parallel methylation of *l*-tryptophan.¹³²



III. Abrine



IV. Gramine

Another simple indole base, gramine, has recently been isolated from the germ of Swedish barley. It is the first alkaloid to be found in any of the *Gramineae*,¹³³ and is identical with donaxine, an alkaloid obtained from an Asiatic reed. The presence of the indole nucleus in gramine or donaxine is apparent not only from the absorption spectrum, but also

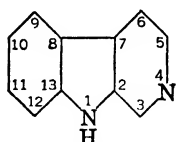
¹³¹ Wieland, Konz, and Mittasch, *Ann.*, **513**, 1 (1934).

¹³² Hoshino, *Ann.*, **520**, 31 (1935).

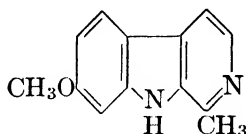
¹³³ von Euler and Erdtman, *Ann.*, **520**, 1 (1935); von Euler, Erdtman, and Hellström, *Ber.*, **69**, 743 (1936).

from the appearance of skatole (3-methylindole) in the zinc dust distillation. A synthesis of gramine has recently been reported.¹³⁴

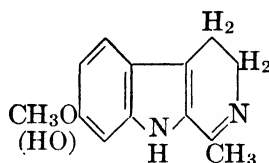
Harmala Alkaloids. The seeds of the African rue, *Peganum harmala*, contain as phosphates the alkaloids harmaline, $C_{13}H_{14}ON_2$; harmine, $C_{13}H_{12}ON_2$; and harmalol, $C_{12}H_{12}ON_2$. The three bases are closely related: harmaline is the methyl ether of harmalol and a dihydro derivative of harmine. The ring system present is a condensation of benzene, pyrrole, and pyridine nuclei, which Perkin and Robinson have designated as 4-carboline.



I. 4-Carboline

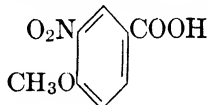
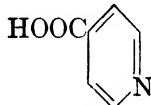


II. Harmine



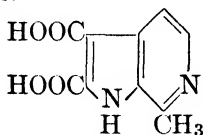
III. Harmaline, Harmalol

Oxidation of harmaline with nitric acid reveals the benzene nucleus, which appears as *m*-nitroanisic acid. In the same reaction, a dibasic acid, $C_{10}H_8O_4N_2$, harminic acid (VI), is formed. In it the pyrrole and pyridine nuclei are contained; on further oxidation it yields isonicotinic acid (γ -pyridinecarboxylic acid).

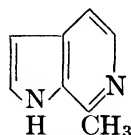
IV. *m*-Nitroanisic acid

V. Isonicotinic acid

In harminic acid the two carboxyl groups derived from the benzene nucleus are adjacent (fluorescein reaction); by decarboxylation one or both may be removed, giving respectively apoharminic acid or apoharmine.



VI. Harminic acid

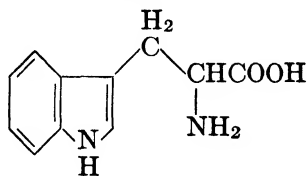


VII. Apoharmine

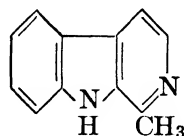
Further evidence for the presence of the pyrrole nucleus in harmaline is found in the formation of red dyestuffs through the action of diazonium salts. The location of the methyl group is deduced from the formation of benzylidene compounds by condensation with benzaldehyde, a reaction characteristic of α -methylpyridines; this leaves, however, two positions (3- and 5-) possible for the methyl group.

¹³⁴ Wieland and Hsing, *Ann.*, **526**, 188 (1936). See, also, Erdtman, *Ber.*, **69**, 2482 (1936); Orechhoff and Norkina, *Ber.*, **68**, 436 (1935).

An important clue to the arrangement of the nuclei in the harmala alkaloids was obtained in the study of harman. This base, which is identical with the alkaloids arabine and loturine, was first prepared by demethoxylation of harmine; it was found to be genetically related to tryptophan, from which it can be obtained by oxidation with ferric chloride in the presence of alcohol.

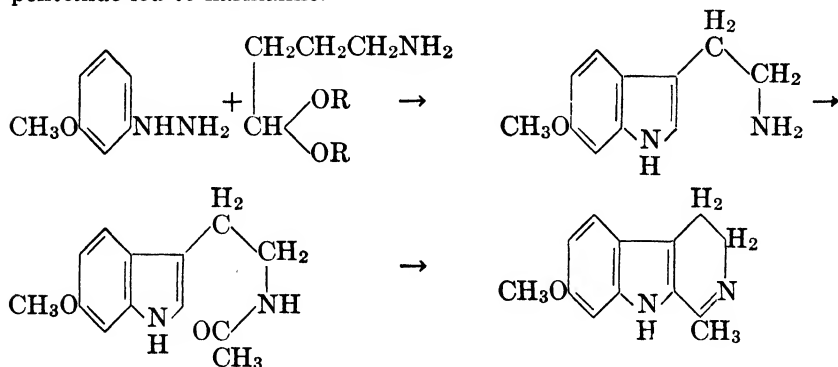


VIII. Tryptophan



IX. Harman

Perkin and Robinson¹³⁵ suggested that the phytochemical synthesis of harmaline proceeds through a condensation of decarboxylated hydroxytryptophan with acetaldehyde, followed by O-methylation and oxidation, considerations that led to the proposal of the 4-carboline arrangement of the three rings. The formation of *m*-nitroanisic acid mentioned above serves to locate the methoxyl group. Various syntheses of harmine, harmaline, and harman have demonstrated the correctness of these conclusions. The harmaline synthesis of Manske, Perkin, and Robinson¹³⁶ in 1927 removed the last point of uncertainty, the location of the alicyclic double bond in harmaline. A simpler synthesis of Späth and Lederer¹³⁷ has as a starting point the condensation of 3-methoxyphenylhydrazine with γ -amino-*n*-butyraldehydediethylacetal. Acetylation of the condensation product and closure of the pyridine ring with phosphorus pentoxide led to harmaline.



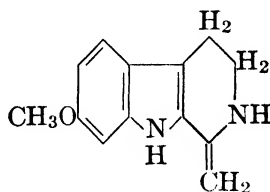
¹³⁵ Perkin and Robinson, *J. Chem. Soc.*, **115**, 933, 967 (1919); Kermack, Perkin, and Robinson, *ibid.*, **119**, 1602 (1921).

¹³⁶ Manske, Perkin, and Robinson, *J. Chem. Soc.*, **1** (1927).

¹³⁷ Späth and Lederer, *Ber.*, **63**, 120, 2102 (1930); Akabori and Saito, *Ber.*, **63**, 2245 (1930).

The phytochemical synthesis of the harman types suggested by Perkin and Robinson has been supported experimentally by G. Hahn, through the preparation of tetrahydroharman from cell-possible substances (tryptamine and acetaldehyde) under physiological conditions (p. 1110).

While harmaline behaves toward alkylating agents like a base with a tertiary pyridine nitrogen atom (formula III), the acetyl derivative and the compounds resulting from the action of benzaldehyde or diazonium salts are probably derived from the tautomeric form X.¹³⁶



X. Harmaline, tautomeric form

Harmine and harmaline have a paralyzing action on the skeletal and cardiac muscles; the use of *Peganum* seeds as a tapeworm remedy probably depends upon paralysis of the musculature of the worm. Harmine has been found identical with banisterine, an alkaloid used in the treatment of Parkinson's disease.

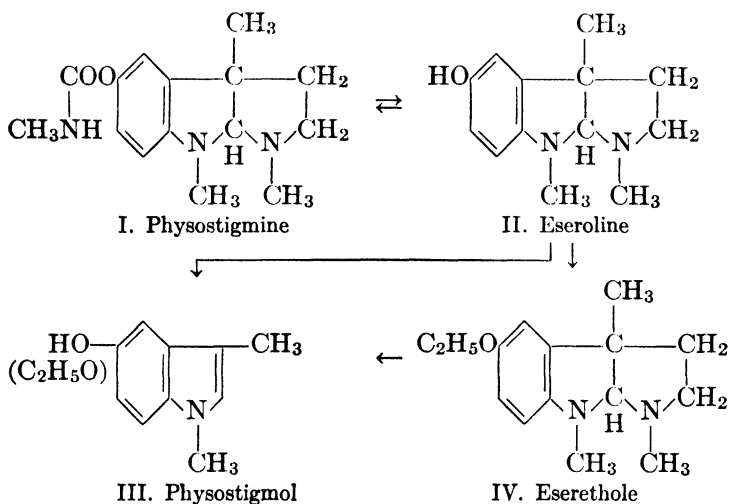
Physostigmine (Eserine). The fruit of the African vine *Physostigma venenosum*, known as the Calabar or Esère bean, is used by the West African natives for the administration of divine justice. An emetic substance in the seed hull often saves the accused person from fatal poisoning. The beans contain several alkaloids, of which physostigmine and geneserine are the most important.

Early investigations of physostigmine, $C_{15}H_{21}O_2N_3$, established the fact that two of the nitrogen atoms are tertiary and carry methyl groups. The third nitrogen is split out as methylamine, together with carbon dioxide, by hydrolysis, and is present in a urethane grouping, a structural feature that has been found in no other alkaloid. The phenolic base resulting from the hydrolysis is known as eseroline (II), and can be converted back to physostigmine by the action of methyl isocyanate.¹³⁸ The ethyl ether of eseroline, known as eserethole (IV), has played an important part in structure determination. From zinc dust distillation of physostigmine, 1- and 2-methylindoles were obtained, but this violent degradation scarcely affords proof of the presence of the indole group.

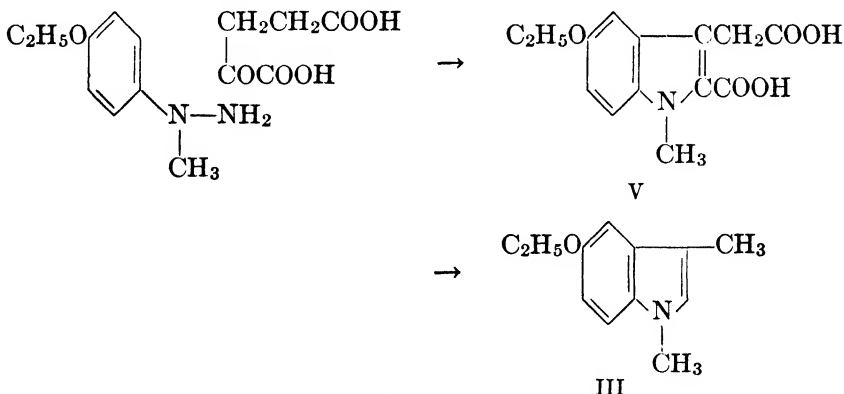
Degradation of eseroline or eserethole methiodides (by heating in an atmosphere of carbon dioxide) results in physostigmol or its ethyl

¹³⁸ Polonovski and Nitzberg, *Bull. soc. chim.*, [4] **19**, 27 (1916).

ether.¹³⁹ Physostigmine still contains the eseroline phenolic hydroxyl group and shows the color reactions characteristic of indoles. The relatively high yield obtained in the degradation indicates that the indole



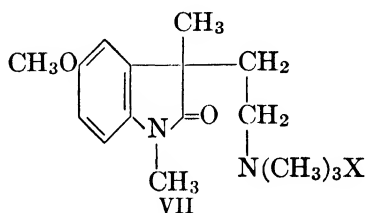
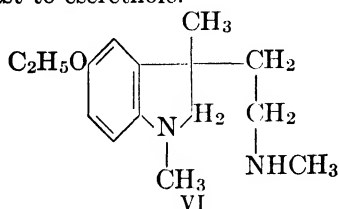
nucleus was already present in eseroline, and therefore in physostigmine. The structure of physostigmol, and hence the position of the eseroline hydroxyl group, was established by Stedman's synthesis of physostigmol ethyl ether.¹³⁹ *p*-Ethoxyphenylmethylhydrazine (from reduction of nitrosomethyl-*p*-phenetidine) was condensed with α -ketoglutaric acid, giving the carboxymethylindoleacetic acid derivative V, from which, on decarboxylation, 5-ethoxy-1,3-dimethylindole, physostigmol ethyl ether (III), was obtained.



¹³⁹ Straus, *Ann.*, **401**, 350 (1913); **406**, 332 (1916). Stedman, *J. Chem. Soc.*, **125**, 1373 (1924).

An alternative synthesis of physostigmol methyl ether by Späth involves condensation of *p*-methoxyphenylmethylhydrazine with propionaldehyde, followed by a Fischer indole ring closure.¹⁴⁰

The physostigmine formula I was advanced by Stedman and Barger¹⁴¹ on the basis of the known structure of physostigmol and the following considerations. Eserethole, on reduction, takes up two hydrogen atoms, which are used in opening a nitrogen-containing ring, for the product, dihydroeserethole (VI), is a secondary amine, in contrast to eserethole.



The further degradation of VI by exhaustive methylation supports the presence of the aminoethyl side chain. Existence of the angular methyl group was conclusively proved by King and Robinson's synthesis and resolution of the methine metho-salts of formula VII, which were identical with those obtained from *l*-esermethole.

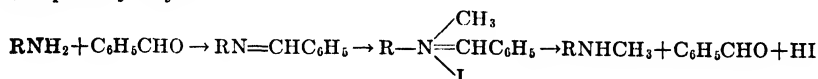
The physostigmine formula received final confirmation in the complete synthesis of Julian and Pikel.¹⁴² This synthesis became feasible through the observation that the hydrogen atom on the 3-carbon of 1,3-dialkyloxindoles is so active that alkylation at this point takes place readily. The desired oxindole derivative was prepared by interaction of *N*-methyl-*p*-phenetidine and α -bromopropionyl bromide, followed by closure of the oxindole ring with aluminum chloride. The ring closure was accompanied by an undesired de-ethylation, therefore the 5-hydroxy-1,3-dimethyloxindole (VIII) was ethylated before further manipulation. The ethoxy compound was condensed with chloroacetonitrile in the presence of sodium ethoxide, and the resulting nitrile (IX) was converted to the amine by catalytic hydrogenation. The primary amine was transformed to the secondary amine (XI) by Decker's method,* and

¹⁴⁰ Späth and Brunner, *Ber.*, **58**, 518 (1925).

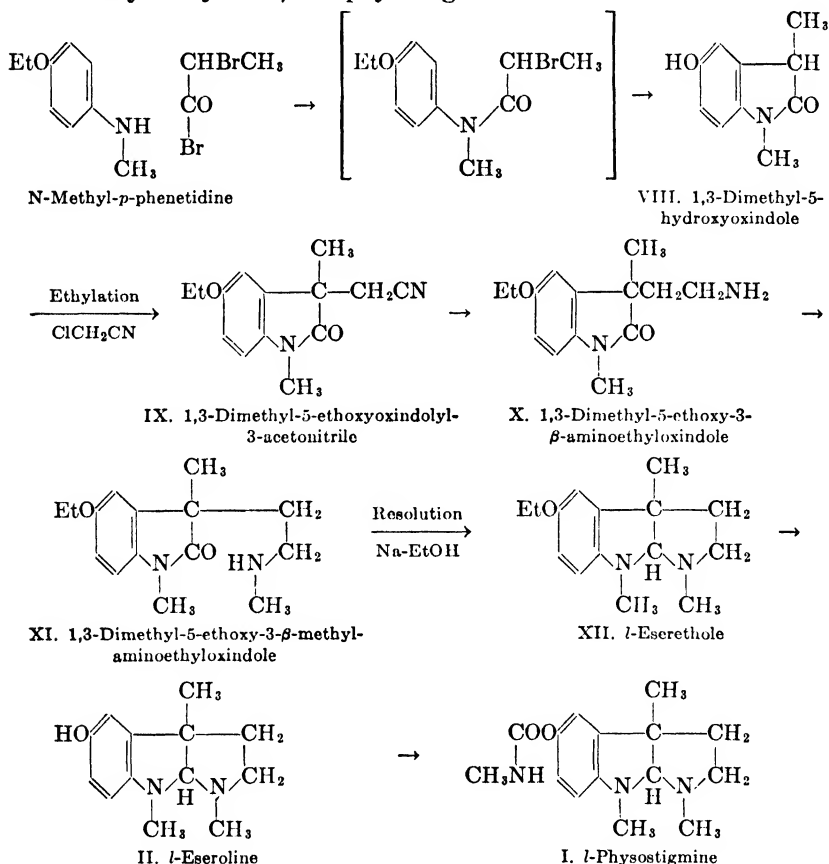
¹⁴¹ Stedman and Barger, *J. Chem. Soc.*, **127**, 247 (1925).

¹⁴² Julian and Pikel, *J. Am. Chem. Soc.*, **57**, 539, 563, 755 (1935).

* Decker's method [Decker and Becker, *Ann.*, **395**, 362 (1913)] for the conversion of primary amines to secondary, consists in condensation of the primary amine with an aldehyde (benzaldehyde), followed by addition of alkyl halide to the Schiff's base and subsequent hydrolysis:



the product was resolved into the *d*- and *l*-isomers at this point, since it was found that resolution at a later stage could not be accomplished. When 1,3-dimethyl-5-ethoxy-3- β -methylaminoethyloxindole (XI) was reduced with sodium and alcohol, ring closure to *l*-eserethole occurred. The *l*-eserethole so obtained could be dealkylated (aluminum chloride) to *l*-eseroline, which was then converted by the Polonovski procedure,¹³⁸ with methyl isocyanate, to *l*-physostigmine.



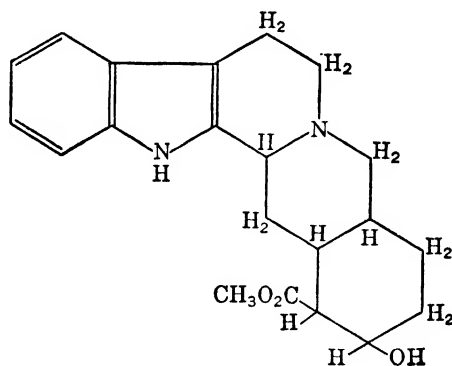
Geneserine, C₁₅H₂₁O₃N₃, contains one oxygen atom more than physostigmine. It can be reduced with ease to physostigmine, and conversely, is formed when physostigmine is treated with hydrogen peroxide, whence its nature as the N-oxide of physostigmine is evident. With the exception of a recently discovered alkaloid of the lupine series,¹⁴³ it is the only natural alkaloid N-oxide that has been found.

¹⁴³ Couch, *J. Am. Chem. Soc.*, **58**, 1296 (1936).

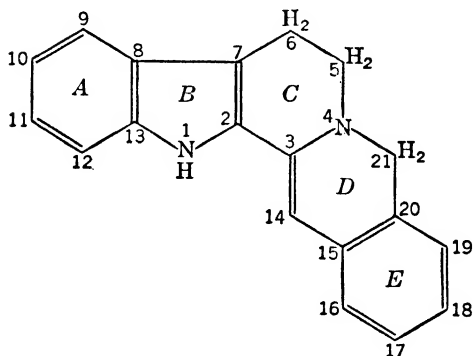
Physostigmine is exceedingly poisonous, the fatal dose for man being in the neighborhood of 10 mg.; death usually results from respiratory paralysis. The alkaloid is used in ophthalmic practice, and especially in the treatment of glaucoma. Geneserine is much less toxic, and is probably converted slowly to physostigmine in the body.

Yohimbine. The bark of the West African tree *Corynanthe yohimbe*, used by the natives as a powerful aphrodisiac, contains a number of related alkaloids, the most important of which is yohimbine. Quebrachine, from quebracho bark (Argentina), is identical with yohimbine.

Yohimbine, $C_{21}H_{26}O_3N_2$, is the methyl ester of yohimbic acid, $C_{19}H_{23}ON_2COOH$. The latter, on decarboxylation, is converted to yohimbol, which still contains the secondary alcoholic hydroxyl group known to be present in yohimbine. When yohimbine is heated with selenium, yobyryne $C_{19}H_{16}N_2$, tetrahydroyobyryne $C_{19}H_{20}N_2$, and keto-dihydroyobyryne $C_{20}H_{16}ON_2$ are obtained. From the fragments obtained



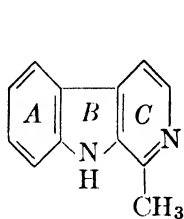
I. Yohimbine.



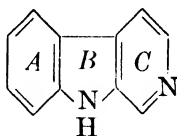
II. Yobyryne.

by degradation of these products, the yohimbine formula I has been derived.*

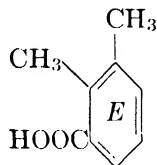
Yohimbic acid, with fused potassium hydroxide or zinc dust, yields harman (scission of ring *D*).¹⁴⁴ Ketodihydroxybyrine, on the other hand, when fused with alkali, suffers breakage of ring *D* at a different point, and gives norharman and 2,3-dimethylbenzoic acid.¹⁴⁵



Harman



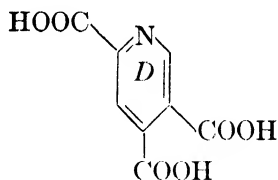
Norharman



2,3-Dimethylbenzoic acid

When tetrahydroxybyrine is oxidized with nitric acid, ring *D* survives intact, and appears as berberonic acid. Ring *D* is likewise found, together with ring *E*, in the form of isoquinoline, from zinc dust degradation of yohimbic acid.¹⁴⁶

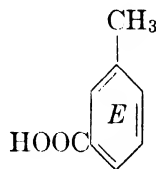
The position of the carboxyl group on ring *E* may be assumed from the formation of dimethylbenzoic acid mentioned above, and from the appearance of *m*-toluic acid when yohimbine or yohimbic acid is treated with superheated steam.



Berberonic acid



Isoquinoline

*m*-Toluic acid

Ring *E* can also be obtained as phthalic acid by oxidation of yobyryne. The indole grouping, rings *A* and *B*, was obtained by Barger and Scholz as 3-ethylindole from potash fusion of yohimbic acid, and ring *A* with ring *B* opened appears in oxalylanthranilic acid, which results from permanganate oxidation of yohimbine.¹⁴⁷

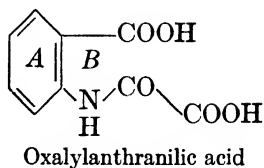
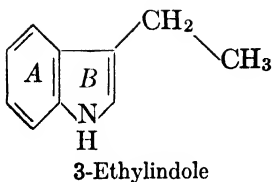
* The position of the alcoholic hydroxyl group is not known with certainty.

¹⁴⁴ Warnat, *Ber.*, **60**, 1118, (1927); Barger and Scholz, *J. Chem. Soc.*, 614 (1933).

¹⁴⁵ Mendlik and Wibaut, *Rec. trav. chim.*, **50**, 91 (1931); Barger and Scholz, *Helv. Chim. Acta*, **16**, 1343 (1933).

¹⁴⁶ Winterstein and Walter, *Helv. Chim. Acta*, **10**, 577 (1927).

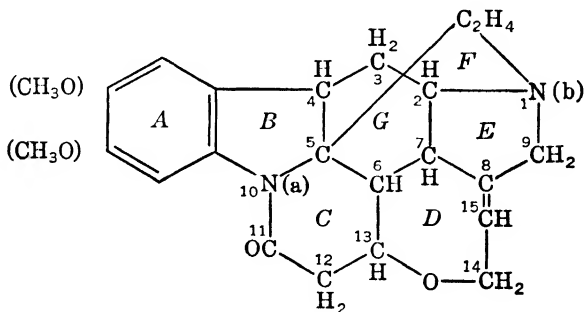
¹⁴⁷ Späth and Bretschneider, *Ber.*, **63**, 2997 (1930).



The position of the yohimbine alcoholic hydroxyl group is still somewhat uncertain. Scholz favors the 14-position, as accounting best for the difficulty observed in the hydrogenation of apoyohimbine, a dehydration product resulting from the action of sulfuric acid on yohimbine. Hahn regards positions-17, -18, or -19 as possible, excluding -14 on the basis of the quantitative alkali degradation of tetrahydroyohimbine into *m*-toluic acid and harman, whereby carbon atom-14 appears as the harman methyl group.¹⁴⁸ In formula I the hydroxyl group has been placed on carbon-17 because the remarkable synthesis of the yohimbine skeleton by Hahn and Werner, accomplished almost entirely under physiological conditions, makes it seem possible that the plant synthesizes the alkaloid by a parallel process, a synthesis that could scarcely succeed were the hydroxyl group at a position other than that shown.¹⁴⁹

Yohimbine is used as an aphrodisiac. It promotes sexual desire in both male and female through dilation of the blood vessels of the genital organs, and also stimulates the sexual centers of the spinal cord.

Strychnos Alkaloids.^{150, 151} The alkaloids of *Strychnos nux-vomica* and of Ignatius beans (*S. Ignatii*), strychnine, brucine, and vomicine, present a structural problem of such complexity that only the salient features can be mentioned. In spite of the intensive researches being carried on at present in the laboratories of Leuchs, Robinson, Wieland,



I. Strychnine (Brucine)

¹⁴⁸ Hahn, Kappes, and Ludewig, *Ber.*, **67**, 686 (1934).

¹⁴⁹ Hahn and Werner, *Ann.*, **520**, 123 (1935).

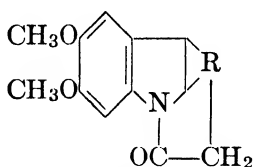
¹⁵⁰ R. Robinson, "Bakerian Lecture," *Proc. Roy. Soc. (London)*, **A130**, 431 (1931).

¹⁵¹ "Annual Review of Biochemistry" (1933), Vol. II, p. 444; (1935), Vol. IV, p. 497.

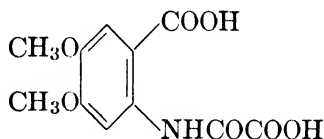
¹⁵¹ Seka, "Alkaloide," Urban and Schwarzenberg, Berlin (1933).

and others, it is not yet possible to present a strychnine structural formula that is certain in every detail. The most recent proposal (I) may serve for discussion; Leuchs favors the linkage of the C_2H_4 group to carbon-3.

Brucine, $C_{23}H_{26}O_4N_2$, is a dimethoxy derivative of strychnine, $C_{21}H_{22}O_2N_2$, and behaves like it in most reactions not involving the aromatic nucleus. The position of the brucine methoxyl groups, already deduced from color reactions, is confirmed by Späth and Bretschneider's oxidation of strychnine to N-oxalyanthranilic acid, of brucine to 4,5-dimethoxy-N-oxalyanthranilic acid (II).

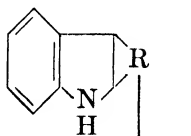


Ia. Brucine

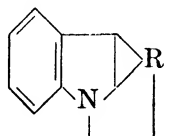


II. 4,5-Dimethoxy-N-oxalyanthranilic acid

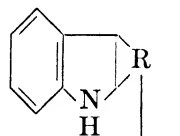
One of the two nitrogen atoms of strychnine, N(a), has no basic properties, and is in a cyclic amido group. By hydrolysis at this point, strychnine is converted into strychnic acid (III). By electrolytic reduction at the amido carbonyl group in strychnine, strychnidine (IV) and tetrahydrostrychnine (V) are formed.



III. Strychnic acid



IV. Strychnidine

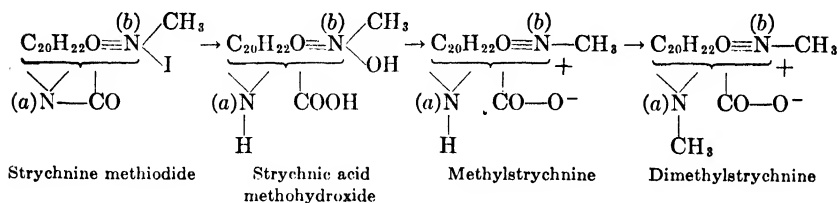


V. Tetrahydrostrychnine

These products still contain a double linkage, whose presence, as in strychnine itself, may be demonstrated by catalytic hydrogenation. The strychnine amido group forms part of the system $R-N(a)-COCH_2-$ in which R is a benzene ring with a free *para*-position; this is deduced from the fact that strychnine, but not strychnidine, condenses with benzaldehyde to give a colored benzylidene derivative. Furthermore, strychnine and strychnidine behave as though related as acylaniline to alkylaniline: strychnine does not give coupling reactions with diazonium salts, but strychnidine yields with diazobenzenesulfonic acid an azo compound that is an indicator resembling methyl orange. The aminostychnidine resulting from reduction

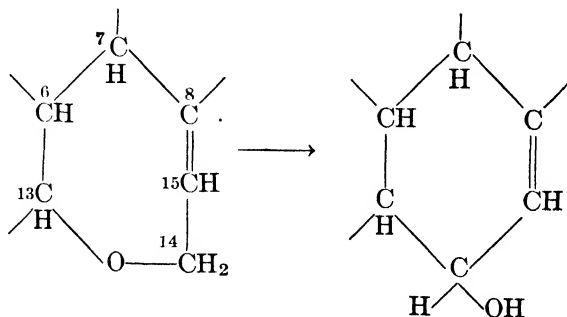
of the azo compound resembles *p*-aminodimethylaniline, and gives analogs of the toluylene dyes.

The second, basic nitrogen atom, N(b), carries no methyl group; it is tertiary, even in hydrogenated derivatives, and therefore is not in an —N=C— group. When strychnine methiodide is treated with alkali or silver oxide, strychnic acid methohydroxide is formed, and this loses a molecule of water to yield the betaine, methylstrychnine, a secondary base [N(a)].

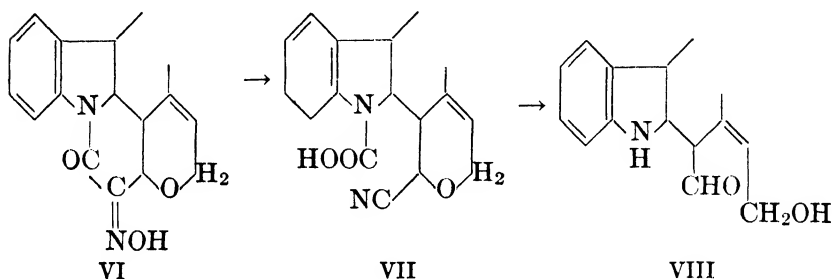


Methylstrychnine reacts with hydriodic acid to give strychnic acid methiodide; with methyl iodide it yields dimethylstrychnine (N-methylstrychnic acid methylbetaine), in which N(a) is tertiary and basic.

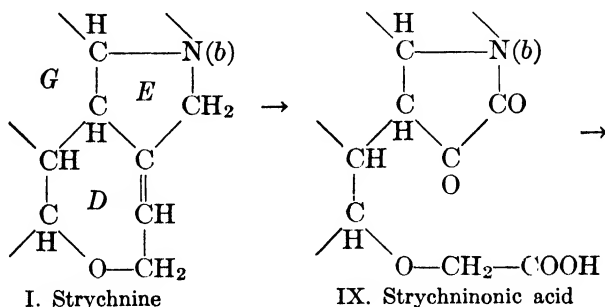
One of the strychnine oxygen atoms is accounted for in the amido carbonyl group; the second oxygen is indifferent to all reagents for the hydroxyl or carbonyl group, and must be present in an ether linkage. By very vigorous reduction, phosphorus and hydriodic acid, the ether oxygen may be eliminated (desoxystrychnine) without loss of any other portion of the molecule, a fact that indicates a cyclic ether structure. Strychnidine does not undergo this type of change. Strychnine and dihydrostrychnine, but not the strychnidines, suffer isomerization under the influence of basic catalysts, giving isostrychnine and dihydroisostrychnine. The members of the *iso*-series contain an alcoholic hydroxyl group, which can be formed only by a change at the ether oxygen; a shrinkage of ring *D* has been suggested to explain the rearrangement. The extreme resistance of the ether oxygen in strychnidine compared



with that in strychnine in the reactions mentioned makes it probable that this oxygen is not far removed from $\text{—N}(a)\text{—CO—}$, as in the system $\text{—N}(a)\text{—CO—CH}_2\text{—CH—O—}$. This arrangement is confirmed by the results from the Beckmann rearrangement of isonitrosostrychnine.¹⁵² Treatment of isonitrosostrychnine hydrochloride with thionyl chloride yields a compound (VII), isomeric with the isonitroso derivative. This rearrangement product hydrolyzes with great ease, losing carbon dioxide and hydrogen cyanide, to give the aldehyde-alcohol VIII, in which one oxygen atom obviously is that of the former cyclic ether structure.*



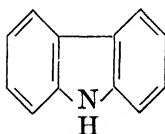
Evidence concerning the propinquity of $\text{N}(b)$, the double linkage, and the ether oxygen has been found in the Leuchs degradation. When strychnine is oxidized with permanganate in acetone, strychninonic and dihydrostrychninonic acids are obtained. Strychninonic acid can be reduced to strychninolic acid, which decomposes in the presence of sodium hydroxide into strychninolone and glycolic acid. In the brucine series the corresponding products obtained are brucinonic and brucinolic acids, and brucinolone. These changes are represented by part-formulas IX–XI.



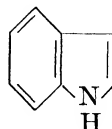
¹⁵² Wieland and Kaziro, *Ann.*, **506**, 60 (1933).

* The ether-linked oxygen is written by Wieland in a 6-membered ring.

(zinc dust distillation) of strychnine. In this reaction rings *A* and *B* are also revealed as indole.

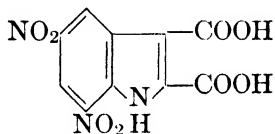


Carbazole

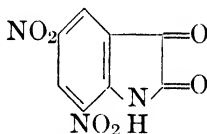


Indole

The indole nucleus has been identified more acceptably through nitric acid oxidation of strychnine. This process leads to dinitrostrycholcarboxylic acid (XIV), which can be further degraded by the Curtius method through the azide and urethane to dinitroisatin (Robinson).

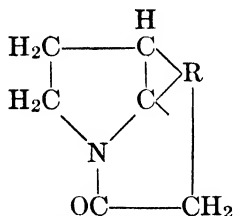


XIV. Dinitrostrycholcarboxylic acid

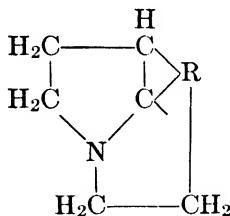


XV. Dinitroisatin

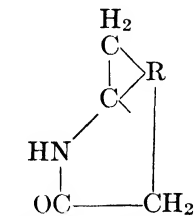
By destruction of four carbon atoms of the aromatic nucleus in strychnine and brucine, derivatives of the hypothetical base nucine are obtained, and similarly from strychnidine and brucidine, derivatives of nucidine. Destruction of the entire aromatic nucleus yields the aponucines or aponucidines.



XVI. Nucine

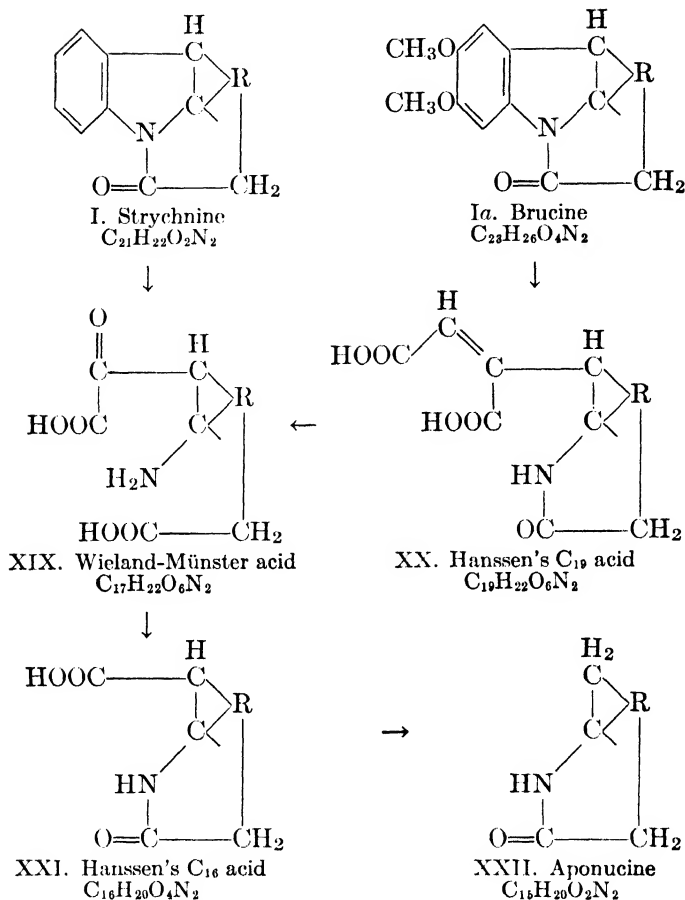


XVII. Nucidine



XVIII. Aponucine

Oxidation of strychnine with chromic acid gives the "Wieland-Münster C₁₇ acid," 2,3-diketoneucine dihydrate. From brucine, "Hanssen's C₁₉ acid" is first obtained, and can be converted to the Wieland-Münster acid. The latter loses a carbon atom with alkaline hydrogen peroxide, and closes the lactam ring, yielding "Hanssen's C₁₆ acid," aponucine-carboxylic acid.



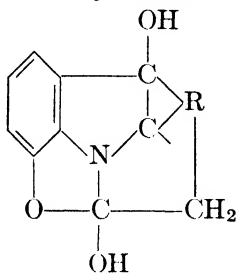
Very little information is available concerning the C_2H_4 chain attached to N(b). It cannot extend to C-4, for evidence on the bromination of diketonucidine shows that C-4 carries a hydrogen atom. The other points in question for the linkage are C-3 and C-5. Leuchs prefers the former, Robinson the latter, on the grounds that strychnine and brucine do not appear to have the properties of dihydroindoles. Union of N(b) and C-5 by an ethylidene group, $—CH—$, would embody



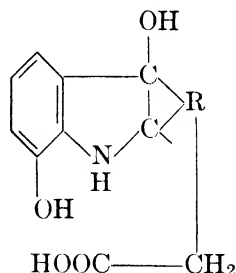
the harmine (p. 1084) skeleton in the strychnine molecule, a point of no little phytochemical interest.

Vomicine, $C_{22}H_{24}O_4N_2$, resembles strychnine and brucine in many of its reactions, but on hydrolysis yields the amino acid, vomicinic acid, which contains a new phenolic hydroxyl group. This phenolic hydroxyl

is likewise generated when vomicine is reduced to vomivicidine by the electrolytic method. Vomicine is therefore represented as containing a benzoxazoline grouping,¹⁵³ but the relationship of the rest of the molecule to that of strychnine is not certain; part-formula XXIII must be regarded as only tentative.



XXIII. Vomicine



XXIV. Vomivicinic acid

Strychnine, because of its extremely high toxicity, is widely used as a pest exterminator. In therapeutic doses it acts to stimulate the respiratory and vasomotor nerve centers; in larger amounts it acts on the spinal cord to cause high reflex irritability, involving all the muscles of the body. Convulsions or tetanus may result from the slightest stimulus, death usually following from suffocation through prolonged contraction of the breathing muscles. Brucine has about one-eighth the toxicity of strychnine.

Ergot Alkaloids.¹⁵⁴ The mycelia of the fungus *Claviceps purpurea*, from diseased rye and other *Gramineae*, constitute the important drug known as ergot. Ergot has yielded, in addition to such simple putrefaction bases as histamine, tyramine, cadaverine, and putrescine, a series of exceedingly complex alkaloids, whose structures are not yet completely elucidated. The seven most important ergot bases are closely related, and may for the most part be discussed as a group. The longest-known pair, ergotinine ($C_{35}H_{39}O_5N_5$) and ergotoxine ($C_{35}H_{41}O_6N_5$), are interconvertible, and differ by a molecule of water (possibly hydrate water). Ergotamine ($C_{33}H_{35}O_5N_5$) and ergotaminine are isomeric, and are likewise interconvertible. Ergonovine ($C_{19}H_{23}O_2N_3$) was discovered during the period 1932–1934 by four independent investigators, who advanced the names ergobasine, ergometrine, ergostetrine, and ergotocin. It is at present believed to be the most active constituent of ergot as regards oxytocic effect. Ergonovine is accompanied by an isomer, ergometrinine, of which it is a transformation product. The fact that in each of these pairs one member is physiologically active (ergotoxine,

¹⁵³ Wieland and co-workers, *Ann.*, **469**, 193 (1929); **491**, 117, 129, 133 (1931).

¹⁵⁴ Barger, "Ergot and Ergotism," Gurney and Jackson, London (1931).

ergotamine, and ergonovine), while the other member is practically inert, suggests that the transformation in each case must involve a similar change in structure. Ergoclavine, $C_{31}H_{37}O_5N_5$ or $C_{25}H_{30}O_4N_4$, has not yet been isomerized.

Early work in the series consisted largely of degradations so violent as to yield little information of value. From destructive distillation of ergotoxine or ergotinine, isobutyrylformamide $(CH_3)_2CHCOCONH_2$ was isolated. Oxidation with permanganate or nitric acid gave benzoic and *p*-nitrobenzoic acids respectively, and a tribasic acid, $C_{14}H_9O_8N$.

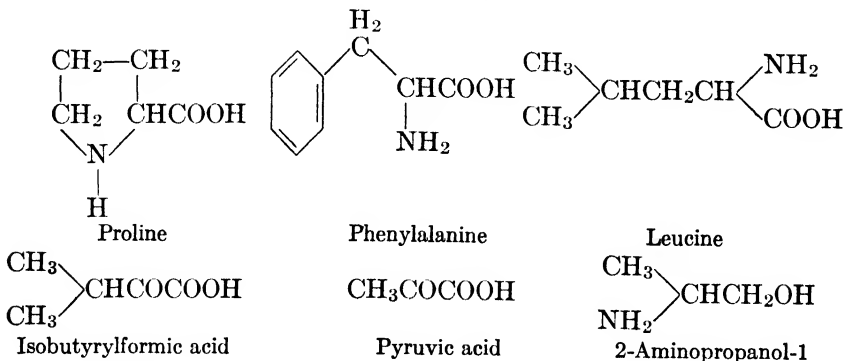
The key to the structure of the ergot group lies in the hydrolysis procedure of Smith and Timmis; ergotoxine, ergotinine, ergotamine, and ergotaminine, on treatment with methyl alcoholic potassium hydroxide, all yield the same product, ergine, of formula $C_{16}H_{17}ON_3$.¹⁵⁵ Ergine is the amide of lysergic acid, and Jacobs and Craig have shown that all the above-mentioned ergot alkaloids can be hydrolyzed to lysergic acid, $C_{16}H_{16}O_2N_2$. This is the only group common to all the members, and the transformations, and changes in physiological action, must be due to changes in the lysergic acid portion of the molecule.

The extensive work of Jacobs and Craig on the hydrolysis of the ergot alkaloids indicates that these are probably constituted as follows:

Ergotinine and ergotoxine consist of lysergic acid, *d*-proline, *l*-phenylalanine, isobutyrylformic acid, and ammonia, probably joined in amide linkages. The presence of the *dextro* form of proline, which occurs elsewhere in nature only in the *levo* form, is especially noteworthy.

Ergonovine and ergometrine are hydroxyisopropylamides of lysergic acid, and give *d*-2-aminopropanol on hydrolysis.^{156, 157}

Ergotamine and ergotaminine are made up of lysergic acid, *l*-phenylalanine, *d*-proline, and perhaps pyruvic acid.



¹⁵⁵ Smith and Timmis, *J. Chem. Soc.*, 763, 1543 (1932); 674 (1934).

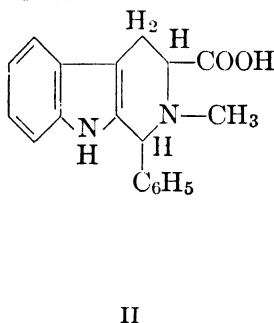
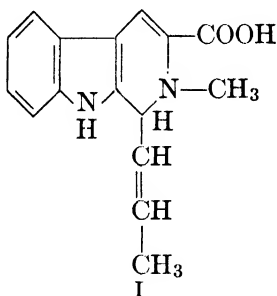
¹⁵⁶ Jacobs and Craig, *Science*, **82**, 16 (1935); *J. Org. Chem.*, **1**, 245 (1936).

¹⁵⁷ Smith and Timmis, *J. Chem. Soc.*, 1166 (1936).

Ergoclavine consists of lysergic acid, ammonia, *l*-leucine, and possibly pyruvic acid.¹⁵⁶

Regardless of the mode of linkage of these constituents to the lysergic acid or ergine portion of the molecule, it is apparent that the constitutional question is essentially that of lysergic acid. Lysergic acid is optically active, contains an N-methyl group but no methoxyl groups, and is monobasic. It forms stable salts with one equivalent of acid. Lysergic acid contains an easily reducible double bond, which must be near the carboxyl group, for dihydrolysergic acid is a much weaker acid and loses carbon dioxide with more difficulty than lysergic acid. Fusion of dihydrolysergic acid with potash yields an indole derivative, probably a methylethylindole or a propylindole. Absorption spectra likewise indicate the presence of the indole nucleus and show that the isolated double linkage is conjugated with it.

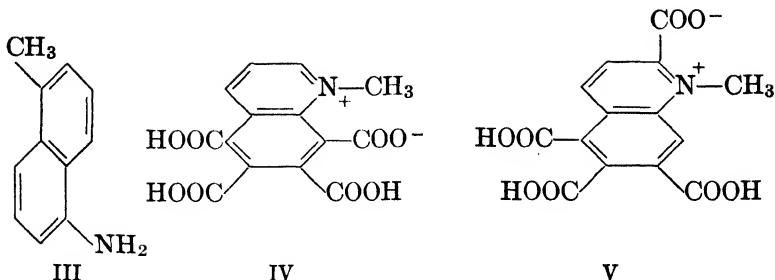
These considerations, among others, and especially the probable genetic relationship of lysergic acid to tryptophan, led to the proposal of the 4-carboline type formula I for lysergic acid.



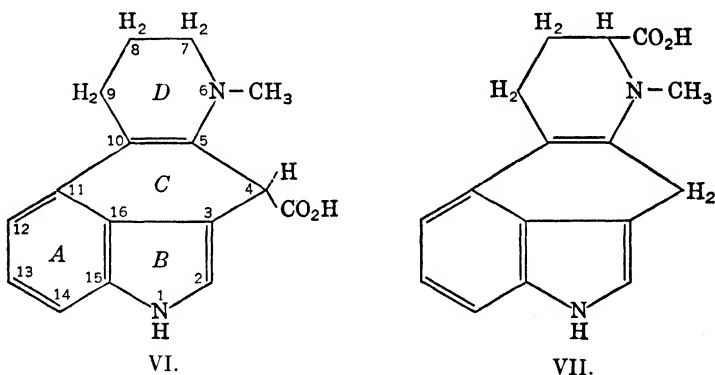
Lysergic acid, however, gives a color test with dimethylaminobenzaldehyde, a reaction characteristic of indoles with the α - or β -positions unsubstituted. Synthetic analogs of I, for example II, prepared by condensation of abrine with benzaldehyde, give no dimethylaminobenzaldehyde test.¹⁵⁸ In the alkali fusion of dihydrolysergic acid, 1-methyl-5-aminonaphthalene (III) is formed. Since, at the same time, methylamine is obtained quantitatively, the amino group in the aminonaphthalene must come from the pyrrole nitrogen atom. The appearance of the aminomethylnaphthalene is difficult to explain on the basis of formula I.

The tribasic acid $C_{14}H_9O_8N$, obtained from nitric acid oxidation of ergotinine or lysergic acid, yields quinoline on destructive distillation. It appears to be an N-methylquinoliniumbetainetricarboxylic acid, IV or V.

¹⁵⁸ Jacobs and Craig, *Science*, **82**, 421 (1935).



These facts have led Jacobs and Craig¹⁵⁹ to advance two formulas for lysergic acid, of which VII is believed to account better for the observed phenomena of isomerism.



Cleavage of ring *B* at 1-2 and 2-3, and of ring *D* at 5-6 and 8-9 accounts for the 1-methyl-5-aminonaphthalene, and cleavage of rings *A* and *B* explains the formation of the acid IV or V.

The unsaturated center of lysergic acid appears to be involved in the isomerization of the ergot alkaloids. Whereas the levorotatory members ergonovine, ergotamine, and ergotoxine exhibit mutarotation, undergoing transformation to the *dextro* members ergometrinine, ergotaminine, and ergotinine, dihydroergonovine does not show mutarotation, nor does dihydrolysergic acid methyl ester, in contrast to the unhydrogenated ester. All the ergot alkaloids yield the same lysergic acid on hydrolysis, but if they are hydrogenated before hydrolysis, the *levo* dihydro alkaloids give a levorotatory acid, α -dihydrolysergic acid, while the *dextro* dihydro alkaloids give a *dextro* acid, γ -dihydrolysergic acid. Lysergic acid itself, on hydrogenation, gives a mixture of the α - and γ -dihydroly-

¹⁵⁹ Jacobs and Craig, *J. Biol. Chem.*, **115**, 227 (1936).

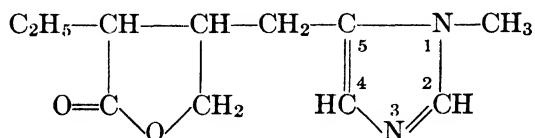
sergic acids, probably as a result of the formation of new asymmetric centers when the double linkage is saturated.

Reductive hydrolysis (sodium and amyl alcohol) of the ergot alkaloids or of lysergic acid methyl ester results in two epimeric alcohols, the α - and β -dihydrolysergols, in which the lysergic acid double linkage has been saturated and the carboxyl group reduced to an alcoholic group. The α - and γ -dihydrolysergic acids, similarly reduced with sodium and alcohol, give α - and γ -dihydrolysergol, respectively. The hypothetical β -dihydrolysergic acid, progenitor of β -dihydrolysergol, is not known, nor is the nature of the isomerism of the three dihydrolysergols clear. On the basis of formula VII for lysergic acid, the isomerism of the pairs of ergot alkaloids can be explained through a shift of the double linkage from 5-10 to 4-5 to 9-10, and reduction of the unsaturation (at 5-10 and at another position) in two configurations might account for the dihydrolysergols.

Ergotoxine, ergotamine, and ergonovine are characterized by their vasoconstrictor action and their power to cause contractions of the uterus. Ergotoxine administration may cause a gangrenous condition of the type observed in the gangrene epidemics (ergotism, St. Anthony's fire) known since the Middle Ages to result from the consumption of bread made with diseased rye.

IMIDAZOLE AND QUINAZOLINE ALKALOIDS: PILOCARPINE AND VASICINE

Jaborandi Alkaloids. The leaves of *Pilocarpus jaborandi* contain several related alkaloids, of which pilocarpine, $C_{11}H_{16}O_2N_2$, is the most important. This alkaloid was shown in the early work of Jowett and of Pinner¹⁶⁰ to be a mono-acid tertiary base containing a lactone group and an imidazole nucleus, but only in recent years has its structure been conclusively demonstrated.

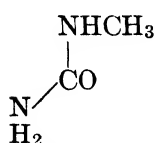


I. Pilocarpine

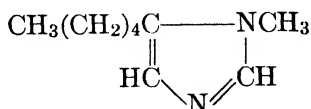
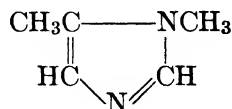
Pilocarpine undergoes isomerization with ease to yield isopilocarpine, a base that also occurs in jaborandi, and that has served for much of the

¹⁶⁰ Jowett, *J. Chem. Soc.*, **83**, 438 (1903); Pinner and Schwarz, *Ber.*, **35**, 192, 2241 (1902).

structural investigation. The arrangement of the two nitrogen atoms is indicated by the appearance of methylurea in oxidations. By distillation of either base with soda-lime, 1-methylimidazole, 1,5-dimethylimidazole, and 1-methyl-5-amylimidazole are obtained.¹⁶¹

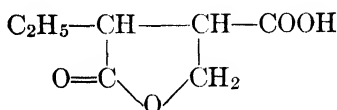


Methylurea

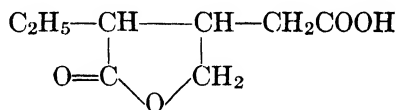
1-Methyl-5-*n*-amylimidazole

1,5-Dimethylimidazole

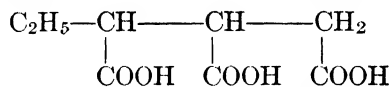
As fragments from the nitrogen-free portion of isopilocarpine, Jowett, by permanganate oxidation, obtained isopilopic and homoisopilopic acids; * the same acids likewise appear in oxidations of pilocarpine as a result of rearrangement.



II. Isopilopic acid



III. Homoisopilopic acid



IV. Ethyltricarballic acid

The constitution of homoisopilopic acid is deduced from the transformation to ethyltricarballic acid by alkali fusion. Chichibabin and Preobrashenski¹⁶² demonstrated the structure of isopilopic acid by synthesis, and prepared the diastereoisomeric pilopic acid corresponding to the nitrogen-free portion of pilocarpine. Pilopic acid undergoes rearrangement with extreme ease to isopilopic acid, so that the latter is always obtained from pilocarpine oxidation. By ozonolysis of pilocarpine and isopilocarpine, isomeric homopilopic acid amides are obtained, showing that the two alkaloids differ only in the stereochemical arrangement of this part of the molecule.¹⁶³

The synthesis of pilocarpine and isopilocarpine was accomplished (1933) by Preobrashenski.¹⁶⁴ *d*-Homoisopilocypyl chloride was con-

¹⁶¹ Akabori and Numano, *Ber.*, **66**, 159 (1933).

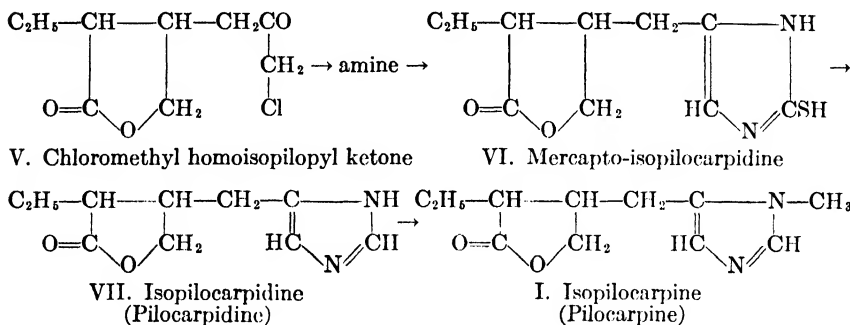
* Originally designated as pilopic and homopilopic acids.

¹⁶² Chichibabin and Preobrashenski, *Ber.*, **63**, 460 (1930).

¹⁶³ Langenbeck, *Ber.*, **57**, 2072 (1924).

¹⁶⁴ Preobrashenski and co-workers, *Ber.*, **66**, 1187, 1536 (1933); **68**, 844, 847, 850 (1935); **69**, 1314, 1835 (1936).

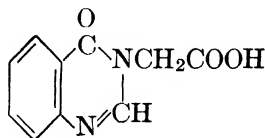
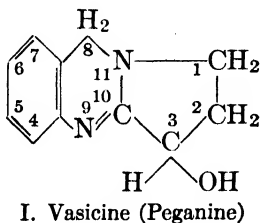
verted by the diazomethane reaction to chloromethyl homoisopilopyl ketone (V). By Gabriel's reaction, V yielded the corresponding amino-methyl ketone, on which the imidazole nucleus was built by heating with potassium thiocyanate. Treatment of the resulting mercapto-isopilocarpidine with ferric chloride gave isopilocarpidine (an isomerization product of the jaborandi alkaloid pilocarpidine), which on methylation yielded isopilocarpine. Pilocarpine was prepared similarly starting from *d*-homopilopie acid.



Pilocarpine acts on the nerve endings of the secretory cells, causing increased secretion of sweat, saliva, and tears. It is used as a diaphoretic, and in optical surgery to cause myosis and to reduce intraocular pressure.

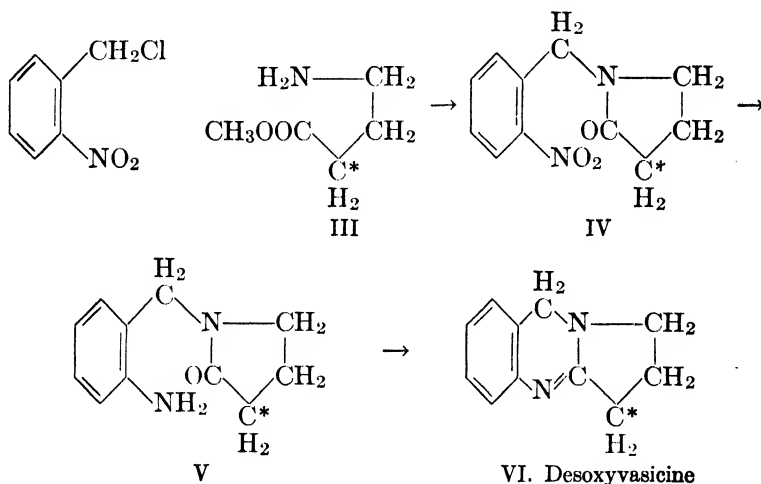
Vasicine. Vasicine, also known as peganine, was first found in the Himalayan plant *Adhatoda vasica*, and was later isolated from the mother liquors from preparation of the harmala alkaloids. *Adhatoda* is used in India as a fish poison, insecticide, and for the relief of asthma. Although vasicine contains an asymmetric carbon atom, it is optically inactive. This phenomenon is encountered rarely in the alkaloid series, and in vasicine the inactive base is formed by racemization during the isolation of the alkaloid. In the plant the base exists in the *levo* form.

The presence of the quinazoline grouping in vasicine, $\text{C}_{11}\text{H}_{12}\text{ON}_2$, was shown by gentle oxidation with permanganate, which resulted in 4-keto-3,4-dihydroquinazolyl-3-acetic acid (II). In this product, $\text{C}_{10}\text{H}_8\text{O}_3\text{N}_2$, only one of the vasicine carbon atoms is missing; this



carbon atom must carry a group that makes it especially susceptible to oxidation. It is indeed the seat of the alcoholic hydroxyl group, whose presence can be shown by gentle acetylation, by the Zerewitinoff reaction (Vol. I, p. 416), and by the chlorination and reduction procedure that leads to desoxyvasicine (p. 99) VI.

The structure of II was easily established by synthesis. For the attachment of the three-carbon chain, one end of which is certainly linked to the 11-position, only C-8 or C-10 come into question. This uncertainty was removed by the synthesis of desoxyvasicine.¹⁶⁵ *o*-Nitrobenzyl chloride was condensed with 4-aminomethylbutyrate to the pyrrolidone IV, which was reduced to the amino derivative (V) and treated with phosphorus oxychloride to close the quinazoline ring.



The alcoholic hydroxyl group of vasicine might be located at positions -2, -3, or -8, the first two being the more probable. The decision in favor of position-3 was reached through Späth's vasicine synthesis, which proceeded like that of desoxyvasicine (formulas III to VI, OH at the starred carbon atom). *o*-Nitrobenzyl chloride was condensed with 4-amino-2-hydroxybutyric acid methyl ester to the pyrrolidone. On reduction of the nitro group, spontaneous ring closure took place to give vasicine.¹⁶⁶

In confirmation of considerations on the possible mode of the phytochemical synthesis of vasicine, Schöpf has prepared desoxyvasicine under physiological conditions (p. 1111).

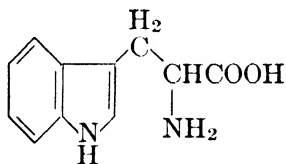
¹⁶⁵ Späth, Kuffner, and Platzer, *Ber.*, **68**, 497 (1935); Hanford and Adams, *J. Am. Chem. Soc.*, **57**, 921 (1935).

¹⁶⁶ Späth, Kuffner, and Platzer, *Ber.*, **68**, 699 (1935).

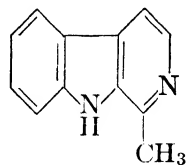
BIOGENESIS OF THE ALKALOIDS

One of the most striking characteristics of an alkaloid-bearing plant is its capacity to produce a number of closely related bases. Examination of any of the series of alkaloids described under various nuclear groups in the preceding pages inevitably suggests that the plant, with its preëminent synthetic ability, has built up such a series from a common parent substance through condensations, methylation, decarboxylation, and oxidation and reduction reactions. This idea was first suggested early in this century by Pictet and by Willstätter, and was put into definite form by Winterstein and Trier (1910) and particularly by Robinson (1917).¹⁶⁷

The amino acids (p. 859), or their transformation products, the amino aldehydes and amines, with formaldehyde, formic acid, and methanol, undoubtedly are the chief building units for the synthesis of alkaloids. The nearer the alkaloid to the parent substance in structure, the more widely it will be found distributed in plants. Hordenine, first found in sprouted barley, probably is formed by N-methylation of tyrosine, and its appearance in the entirely unrelated *Anhalonium* cactus (anhaline, p. 1064) is not surprising. Arabine and loturine are identical with harman, the framework of the harmala alkaloids. The close relationship of harman to tryptophan or tryptamine accounts plausibly for the presence of the harman grouping in three unrelated plant families.



Tryptophan



Harman

Individual species or families, on the other hand, may possess a characteristic ability to cause condensations and ring enlargements leading to the synthesis of alkaloids peculiar to the species.

The first experimental demonstration of the simplicity of method by which the plant may synthesize alkaloid structures was Robinson's condensation of succinic aldehyde and methylamine with acetonedicarboxylic acid (p. 1050). This pioneering experiment stands out in sharp contrast to the involved and laborious synthesis of tropinone by the

¹⁶⁷ Robinson, *J. Chem. Soc.*, **111**, 876 (1917).

classical laboratory methods, and it furnished the stimulus for the present activity in alkaloid synthesis under physiological conditions.

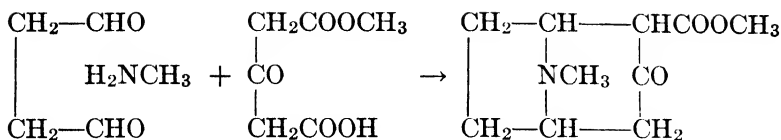
According to Schöpf,¹⁶⁸ synthesis in the plant cell may take place with participation of specific enzyme systems, adapted to the production of one definite substance, as for example the synthesis of starch from carbon dioxide; or unspecific enzymes may take part, such enzymes as have a general function, as decarboxylation, hydrogenation, dehydrogenation, and oxidation; or finally, natural products, or the intermediates from which they are derived, may be formed without the participation of enzymes, when sufficiently reactive units arise together in the course of cell metabolism. This last case is susceptible of study in the laboratory. Essential conditions to be observed are hydrogen-ion concentrations and temperatures comparable to those under which the plant works, as well as the use of starting materials that the plant may be expected to have available.

There can be no doubt as to the ability of the plant to reduce ketone groups and to accomplish esterification, so that the question of the phytochemical synthesis of the alkaloids of the belladonna group, the tropine derivatives, is largely that of the synthesis of tropinone. Succinic aldehyde (from degradation of ornithine), methylamine, and acetonedicarboxylic acid are all cell-possible substances, but Robinson's synthesis is open to objections, because the condensation leading to tropinonedicarboxylic acid was carried out in strongly alkaline solution, and the subsequent decarboxylation required physiologically impossible conditions. When, however, the condensation is accomplished in buffered solution (0.04 molar) between pH 3 and pH 11 at 25°, spontaneous decarboxylation takes place, and tropinone is obtained in excellent yields. The same mechanism may be imagined to operate in the formation of pseudopelletierine (p. 1034), the ring homolog of tropinone, in the plant. Here glutaric aldehyde, conceivably arising in the cell from the degradation of lysine, $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{COOH})\text{NH}_2$, takes the place of succinic aldehyde. By condensing glutaric aldehyde with methylamine and acetonedicarboxylic acid in solutions buffered to pH 7 at 25°, Schöpf was able to prepare pseudopelletierine in nearly quantitative yield.¹⁶⁹

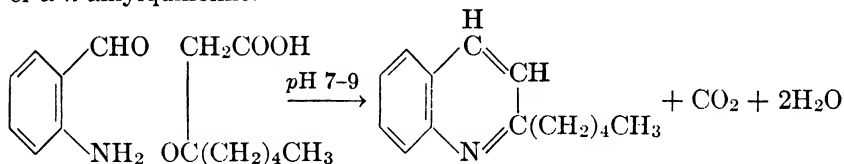
The cocaine types, derived from ecgonine, demand the retention of one carboxyl group during the condensation. This, also, can be accomplished under physiologically possible conditions (pH 5), if the monomethyl ester of acetonedicarboxylic acid is used.

¹⁶⁸ Schöpf, *Ann.*, **497**, 1 (1932).

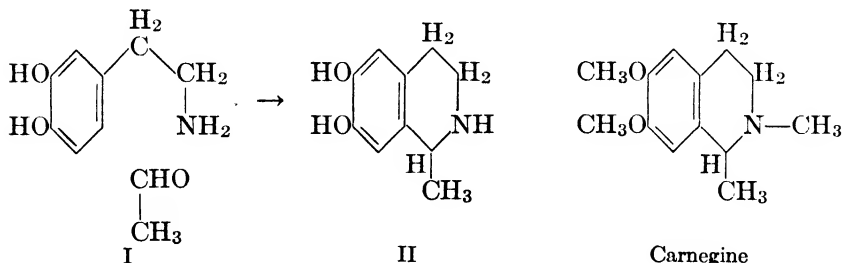
¹⁶⁹ Schöpf and Lehmann, *Ann.*, **518**, 1 (1935).



The alkaloids containing a quinoline nucleus could reasonably be supposed to be formed through a Friedländer synthesis, condensation of *o*-aminobenzaldehyde with ketones. The oxidation product of *o*-aminobenzaldehyde, namely anthranilic acid, is found frequently in nature, and is observed as a degradation product from tryptophan in the animal body. Methyl ketones are present in many ethereal oils. The Friedländer condensation, which would lead to the quinoline alkaloids of the angostura type, proceeds, however, only in alkaline solution, at pH 11-12. The biosynthesis of the quinoline group appears to have its starting point in substances that may be regarded as the progenitors of the methyl ketones, namely the β -keto acids. The synthesis of one of the members of the angostura alkaloids (p. 1062) illustrates this sufficiently. Condensation of very dilute solutions of *o*-aminobenzaldehyde and caproylacetic acid at 25° and pH 7-9 resulted in an excellent yield of α -*n*-amylquinoline.¹⁷⁰



The substituted phenylethylamines have long been considered as the probable parent substances of the extensive group of isoquinoline alkaloids. Most of these alkaloids carry groups in the 5- and 6-positions and might be formed from condensation of the appropriate aldehyde with the dihydroxyphenylethylamine (I), arising from degradation of dihydroxyphenylalanine. The reaction with acetaldehyde, for example, takes place readily at ordinary temperatures in the pH range 3-5,

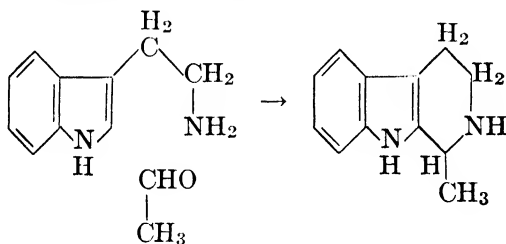


¹⁷⁰ Schöpf and Lehmann, *Ann.*, **497**, 7 (1932).

to give the tetrahydroisoquinoline derivative (II), which is a demethylated analog of the alkaloids carnegine and salsoline.¹⁷¹

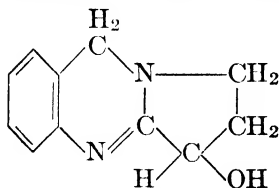
The fact that carnegine and salsoline occur naturally as the racemic forms makes it seem probable that they are synthesized in a similar way, and not under the influence of enzymes. The condensation under physiological conditions has been extended by Hahn to the synthesis of benzyloisoquinoline bases of the laudanose type.¹⁷²

For the ever-increasing group of alkaloids containing the indole nucleus, the building unit must be tryptamine, derived from tryptophan by decarboxylation. In this series, too, the mechanism has been subjected to direct investigation. Tryptamine reacts with acetaldehyde at ordinary temperatures, and at pH 5-7, to give tetrahydroharman.¹⁷³

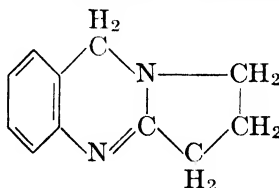


The reaction does not proceed satisfactorily with more complicated aldehydes, but succeeds with α -keto acids, which are probably the biochemical progenitors of the aldehydes. It is interesting to note that the condensation is accelerated markedly by sunlight. The problem of decarboxylation under physiological conditions of the condensation products from the α -keto acids has not been solved, but Hahn has used the method for the synthesis of the complicated skeleton present in the yohimbine group.¹⁴⁹

The synthetic methods outlined above have recently been applied to the construction of another complex nucleus, that of vasicine (III). The quinazoline system present in vasicine may be imagined as arising from interaction of *o*-aminobenzaldehyde and α -hydroxy- γ -aminobutyric



III. Vasicine



IV. Desoxyvasicine

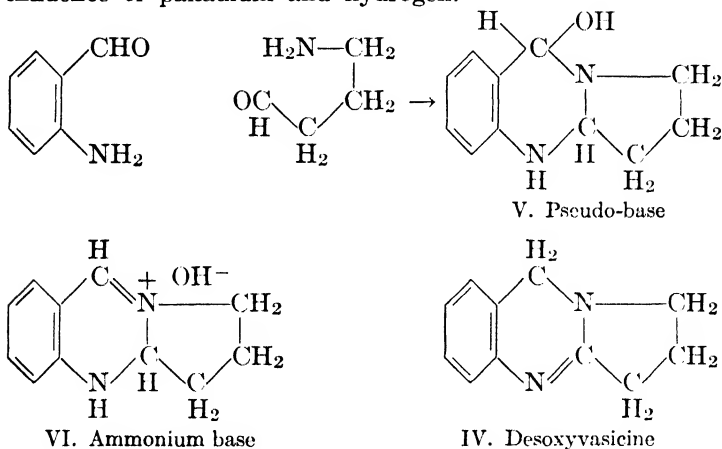
¹⁷¹ Schöpf and Bayerle, *Ann.*, **513**, 190 (1934).

¹⁷² Hahn and Schales, *Ber.*, **68**, 24 (1935).

¹⁷³ Hahn and Ludewig, *Ber.*, **67**, 2031 (1934).

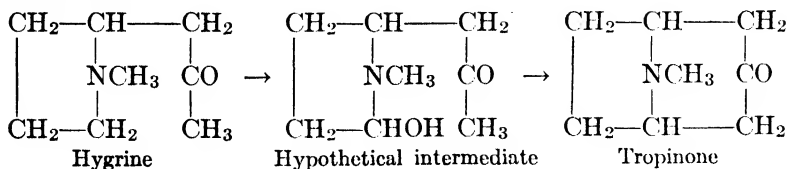
aldehyde, followed by isomerization and shift of two hydrogen atoms. Unfortunately, α -hydroxy- γ -aminobutyraldehyde is not known, but γ -aminobutyraldehyde, in the form of the diethylacetal, is available, and the synthesis of desoxyvasicine (IV) by the use of this aminoaldehyde makes the above hypothesis of the biogenesis of vasicine seem reasonable.

In dilute solution, at pH 5, γ -aminobutyraldehydediethylacetal undergoes rapid hydrolysis and the liberated aldehyde condenses with *o*-aminobenzaldehyde to the pseudo-base V. The pseudo-base isomerizes to the colored quaternary ammonium base VI, in which a shift of two hydrogen atoms to the quinazoline formula of desoxyvasicine takes place under the influence of palladium and hydrogen.



If this process represents the biosynthetic course of vasicine formation, the last step, hydrogen shift, probably takes place in the plant through the action of enzymes.¹⁷⁴

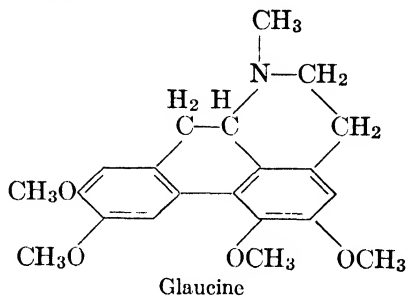
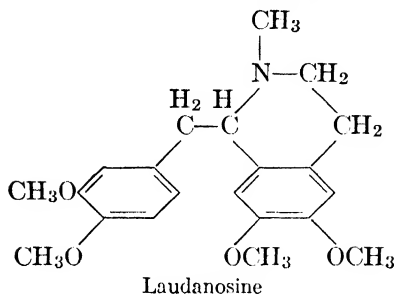
Several other syntheses under similar conditions, as for example that of hygrine¹⁷⁵ and of lobelanine,¹⁶⁹ have been accomplished. Attempts to verify inviting theoretical relationships through oxidation or dehydrogenation reactions have not been successful. The conversion of hygrine to tropinone, which might be expected to proceed as follows, failed:



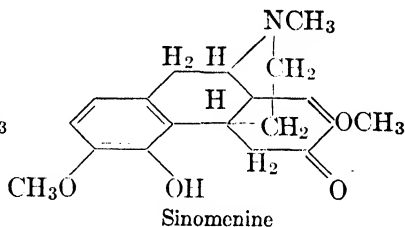
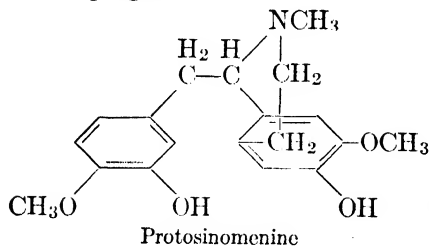
¹⁷⁴ Schöpf and Oechler, *Ann.*, **523**, 1 (1936).

¹⁷⁵ Robinson, *J. Chem. Soc.*, 1079 (1936).

The formal relationship existing between alkaloids of the benzyloquinoline type and those of the aporphine or morphine series, as for example laudanosine and glaucine, has led to fruitless attempts to establish the missing linkage in such types.



The hypothetical progenitor of the sinomenine series, protosinomenine, has been synthesized, and experiments on its conversion to sinomenine are in progress.¹⁷⁵



In spite of the disappointments mentioned, the success of the biosynthetic methods described is inspiring. It can be predicted that, with refinements of technique and choice of more suitable reactants, syntheses of this type can be extended to afford a great deal of additional information on the probable mechanism of formation of the alkaloids in the plant.

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CHAPTER 13

THE ANTHOCYANINS AND THE FLAVONES

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THE ANTHOCYANINS

Introduction. The pigments of plants are roughly divisible into two major classes.^{1,2,3,4,5} The plastid pigments represent one group.⁴

¹ Rupe, "Die Chemie der natürlichen Farbstoffe," Vieweg and Sohn, Braunschweig (1900), Vol. I; (1909), Vol. II.

² Mayer, "Chemie der organischen Farbstoffe," 3rd ed., Springer, Berlin (1935), Vol. II, pp. 134-150.

³ Perkin and Everest, "The Natural Organic Colouring Matters," Longmans, Green and Co., London (1918).

⁴ Wheldale, "The Anthocyanin Pigments of Plants," University Press, Cambridge (1916).

⁵ Karrer, "Handbuch der Pflanzenanalyse," edited by Klein, Springer, Vienna (1932), Vol. III, pp. 941-984.

They are associated with the protoplasmic structure of the plant. The second group consists of those pigments which generally exist in solution in the cell sap. These pigments belong to a group of glycosides designated as "anthocyanins."^{3,4*} The innumerable shades of blue, purple, violet, mauve, and magenta and nearly all the reds which appear in flowers, fruits, leaves, and stems of plants are due to anthocyanin pigments. The sugar-free pigments or aglucons, are called *anthocyanidins*.⁴ Although it is held that the anthocyanins are usually dissolved in the cell sap, they can also occur in an amorphous or crystalline state as in the *Delphinium* spp., *Passiflora* spp., *Rubus* spp., and others.

The pioneer researches of Willstätter and his students^{6,7,8} made it clear that the numerous individual anthocyanins contain similar nuclei. The wide variations in color are due to slight alterations in the molecule which do not affect the basic molecular skeleton. Thus, the anthocyanins represent a chemical class of natural products, in the same sense as the fats, carbohydrates, or proteins represent distinct classes.

The Basic Structure of the Anthocyanins. Willstätter's success^{6,7} with these plant coloring matters was to a large extent due to his early recognition of their amphoteric nature. They are capable of forming salts with both acids and bases. The salts formed with acids were first recognized as oxonium salts of the type known as flavylum salts. The methods of isolation and purification employed with these pigments rest on this basis.

The fundamental parent substance of the entire group is the heterocyclic nucleus known as benzopyrylium chloride (I) discovered by Decker and von Fellenberg⁹ which they formulated on the basis of the oxonium theory.† By substituting a phenyl residue in position 2

*The term "anthocyan" is derived from the Greek roots signifying respectively "flower" and "blue." It was introduced by the botanist Marquart in 1835 to designate the blue pigments of flowers. Shortly thereafter the belief developed that the red and blue pigments were merely different forms of the same substance and that their different colors were due to variations in the character of the cell sap; consequently, the use of the term was extended to include all the soluble pigments of this group. When it was learned that these pigments are always combined with sugars, and thus occur as glycosides, the ending "in" was attached.

⁶ Willstätter, *Sitzber. preuss. Akad. Wiss.*, **29**, 402, 769 (1914); also *Ber.*, **47**, 2865 (1914).

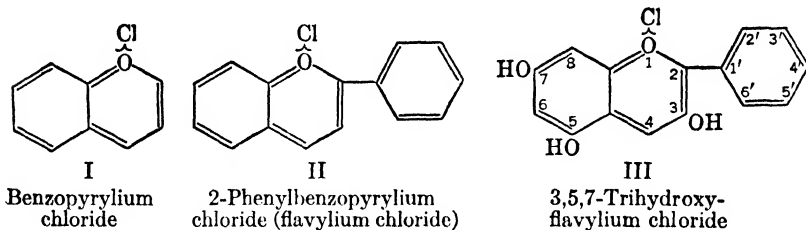
⁷ Willstätter and co-workers, *Ann.*, **401**, 189 (1913); **408**, 1, 15, 42, 61, 83, 110, 147 (1915); **412**, 113, 137, 149, 164, 178, 195, 217, 231 (1916); *Ber.*, **57**, 1938, 1945 (1924).

⁸ Robinson, *Naturwissenschaften*, **20**, 612 (1932). Summary of Professor R. Willstätter's investigations on the anthocyanins.

⁹ Decker and von Fellenberg, *Ann.*, **364**, 1 (1908).

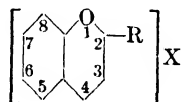
†The oxonium theory as a whole possesses certain disadvantages. Although the quadrivalency of oxygen may be defended on the basis of the electronic theory of valency, it cannot be regarded as acceptably proved. Recent work has shown that the amphoteric nature of the benzopyrylium salts and their behavior on oxidation can in many respects be more adequately defined through another theoretical approach, the carbonium and

of the benzopyrylium chloride (I), 2-phenylbenzopyrylium chloride or flavylum chloride (II) is obtained. The placement of hydroxyl groups in positions 3, 5, and 7 yields 3,5,7-trihydroxyflavylium chloride (III) the simplest intact structural unit of the anthocyanins. In fact, all the members of this group found to date can be regarded as polyhydroxy-2-phenylbenzopyrylium salts.



The Type Group of the Anthocyanidins. The investigations of Willstätter,^{6,7,8} Karrer,⁵ and Robinson^{8,10,11,12,13} have shown that there are six type groups of the anthocyanins to which the various individuals can be referred.* These groups are known respectively as pelargonidin (IV), cyanidin (V), delphinidin (VI), peonidin (VII), malvidin † or syringidin (VIII), and hirsutidin (IX). It is to be observed that pelargonidin (IV), cyanidin (V), and delphinidin (VI) are the fundamental types of the class, whereas peonidin (VII) is a monomethyl ether of cyanidin and malvidin (syringidin) (VIII) and hirsutidin (IX) are respectively the di-, and trimethyl ethers of delphinidin. All the type groups have been synthesized by Robinson and his co-workers

carbenium theories. A detailed consideration of the evidence for and against any one theory is beyond the scope of this chapter. The reader is referred to the recent review article by Hill [*Chem. Rev.*, **19**, 27 (1936)] where the properties, synthesis, and structure of the benzopyrylium salts are considered in the light of the various theories. In the review by Hill the benzopyrylium (chromylum) salts are represented by the following general formula:



¹⁰ Robinson, *Nature*, **137**, 94 (1936); *Ber.*, **67A**, 85 (1934).

¹¹ Robinson *Nature* (Royal Jubilee Number), **135**, 732 (1935).

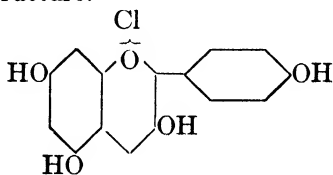
¹² Robinson, *J. Soc. Chem. Ind.*, **52**, 737 (1933).

¹³ Robinson, President's Address, Section B, Chemistry, British Association for the Advancement of Science; reprinted in *Nature*, **132**, 625 (1933).

* The classes are usually designated by root names derived from the Latin botanical nomenclature.

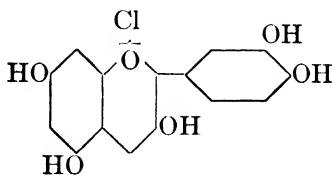
† Malvidin is also called syringidin since it yields syringic acid on degradation with dilute alkali.

through methods that leave no doubt as to the validity of their structure.*



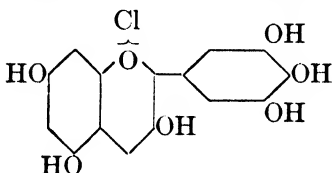
IV

Pelargonidin
[3,5,7,4'-tetrahydroxy-2-phenylbenzopyrylium chloride]



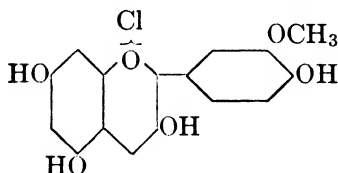
V

Cyanidin
[3,5,7,3',4'-pentahydroxy-2-phenylbenzopyrylium chloride]



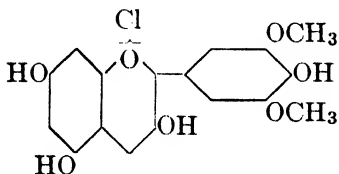
VI

Delphinidin
[3,5,7,3',4',5'-hexahydroxy-2-phenylbenzopyrylium chloride]



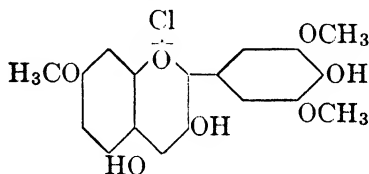
VII

Peonidin
[3,5,7,4'-tetrahydroxy-3'-methoxy-2-phenylbenzopyrylium chloride]



VIII

Malvidin (syringidin)
[3,5,7,4'-tetrahydroxy-3',5'-dimethoxy-2-phenylbenzopyrylium chloride]



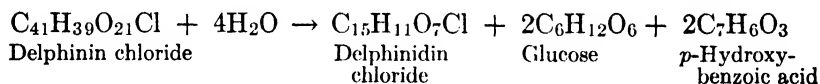
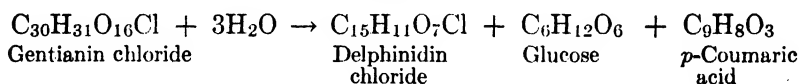
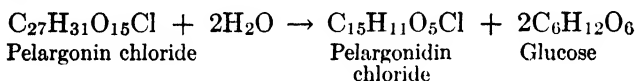
IX

Hirsutidin
[3,5,4'-trihydroxy-7,3',5'-trimethoxy-2-phenylbenzopyrylium chloride]

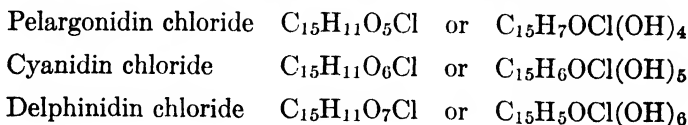
The Glycosidic Nature of the Anthocyanins.^{5,8,11,12,13} All the members isolated so far yield in addition to the *anthocyanidin* a sugar (p. 1454), or several sugars, when boiled for a short time with dilute mineral acids. The greater number of the anthocyanins fall into a comparatively restricted number of categories with the sugar residues attached to the 3, or 3,5 hydroxyls; thus (a) the 3-monoglucosides and 3-monogalactosides; (b) the 3-rhamnosides and other 3-pentosides; (c)

* It is a remarkable fact that almost the whole range of anthocyanin pigments of flowers, fruits, and blossoms is derived from the three fundamental anthocyanidin types, (IV), (V), (VI), by various substitutions in the hydroxyl group. However, some exceptions exist. The bluest anthocyanins in the beet, for instance, originally isolated by Willstätter, are nitrogenous pigments (see Robison and Robinson, *J. Chem. Soc.*, 1439 [1932]; 25 [1933]; 446, 449, 453 [1937].

the 3-biosides; (d) the 3,5-diglucosides; and (e) the acylated anthocyanins. The anthocyanins of class (d) are the most widely distributed and best-known members of the group. Pelargonin, the 3,5-diglucoside of pelargonidin (IV), the pigment of the scarlet pelargonium and possibly the first member of the soluble pigments to be obtained in a crystalline condition,^{7,8,10} belongs to this group, as does cyanin, isolated by Willstätter in 1914 from the cornflower. Pelargonin, cyanin, peonin, malvin, and hirsutin have been synthesized by Robinson.¹⁰ Certain anthocyanins, delphinin, gentianin, monardaein, and salvianin, for instance, yield in addition to the pigment, and the sugar or sugars, a third component which is invariably an organic acid. These represent the acylated anthocyanins [group (e) above]. The acids found so far are *p*-hydroxybenzoic, malonic, *p*-hydroxycinnamic, and *p*-coumaric acid (see the work of Karrer^{5,14}). The acid radicals can be either in ester combination with one of the hydroxyl groups of the pigment nucleus, or attached to an hydroxyl of the sugar component. The hydrolysis of some representative anthocyanins is illustrated in the following equations:



The Degradation Products of the Anthocyanidins.^{5,7,14} The occurrence of the 2-phenylbenzopyrylium nucleus (II) in the various anthocyanins was originally established by Willstätter through an alkaline fusion of the sugar-free pigments.* When the empirical formulas of the three parent classes are compared, it becomes evident that they differ from each other by a single oxygen atom, as represented below:

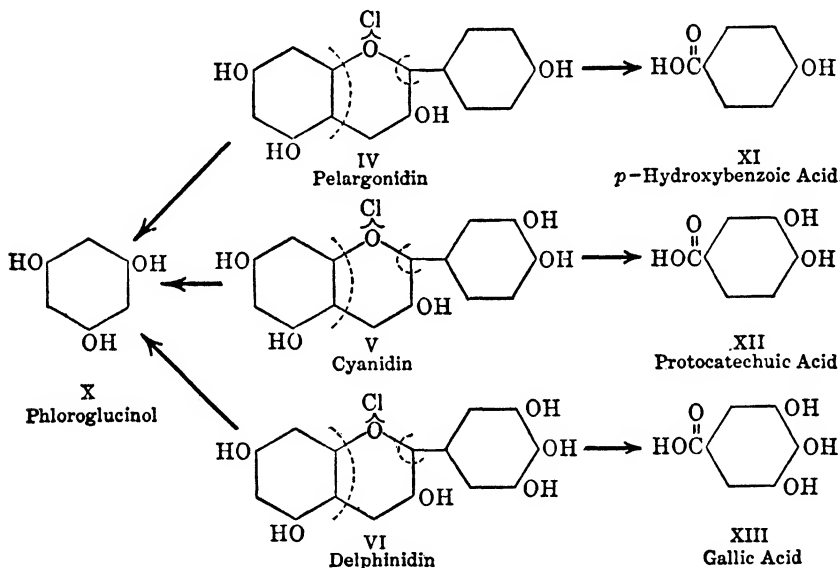


¹⁴ Karrer and co-workers, *Helv. Chim. Acta*, **10**, 67, 729 (1927); **12**, 292 (1929); **15**, 507 (1932).

* Supplementary evidence which indicated that the anthocyanidins contain the 2-phenylbenzopyrylium nucleus was the reduction (*in vitro*), of the flavonol quercetin (XXIV) to cyanidin (XXV).⁶

These three anthocyanidins degrade upon fusion with potassium hydroxide into two simple products, one of which is a phenol, the other a phenol-carboxylic acid. The phenol obtained from each of the three homologous anthocyanidins is the same, namely, phloroglucinol (1,3,5-trihydroxybenzene) (X). The phenolcarboxylic acid obtained from the simplest anthocyanidin, pelargonidin (IV), is *p*-hydroxybenzoic acid (XI); that from the next simplest, cyanidin (V) is 3,4-dihydroxybenzoic acid or protocatechuic acid (XII); that from the third, delphinidin (VI), is 3,4,5-trihydroxybenzoic acid or gallic acid (XIII).

The relationship of the phenol common to the three parent anthocyanidins and the respective phenolic acids is illustrated in the scheme below:



Methods introduced by Paul Karrer in 1927^{5,14} for the purpose of establishing the precise nature of the phenyl residue in position 2 and the points of linkage of the sugar residues have proved fruitful and reliable. Prior to Karrer's work the position of the methoxyl residues in the anthocyanidin groups VII, VIII, and IX was not known, since the concentrated alkali employed to degrade the pigments also removed the methoxyls. The position of the sugar residues was likewise an open question. Karrer's degradation of the sugar-free pigments with dilute barium or sodium hydroxide (10 per cent) in an atmosphere of hydrogen, which yielded the phenolic acid with the methoxyl groups intact, was therefore, a significant advance. The results obtained by this method

were confirmed through a second method wherein a degradative oxidation with hydrogen peroxide was first employed to open the ring of the anthocyanidin between carbon atoms numbers 2 and 3 without removing either the sugar residue or the methoxyl groups. The resulting intermediate, which was obtained from malvin,* could subsequently be quantitatively hydrolyzed with dilute acid or alkali to the corresponding methoxylated phenolic acid, e.g., syringic acid. The derivative of phloroglucinol, which might be formed in this hydrolysis from the various methoxylated anthocyanidins and which would contain one of the sugar residues and an acid side-chain, has so far not been isolated in a crystalline condition.

The course of these degradations can be illustrated with the diglucoside, malvin chloride (XX). Starting with malvidin chloride (VIII), degradation with dilute alkali gave on the one hand phloroglucinol (X) and syringic acid (XV) or 3,5-dimethoxygallic acid. The oxidative degradation with hydrogen peroxide transformed malvin chloride (XX) into an intermediate malvon, whose exact constitution is not known, but which may be represented either by the structure XIVa or XIVb.

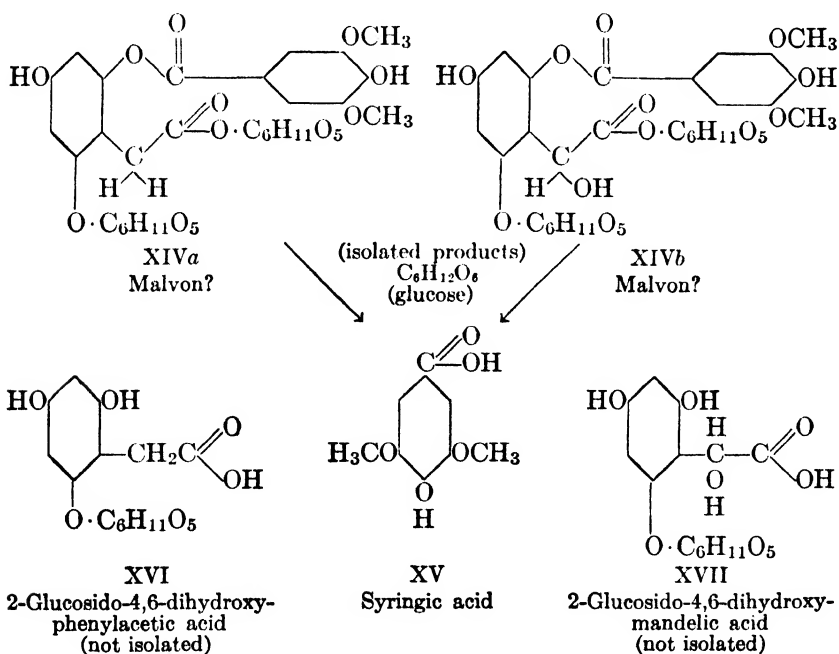
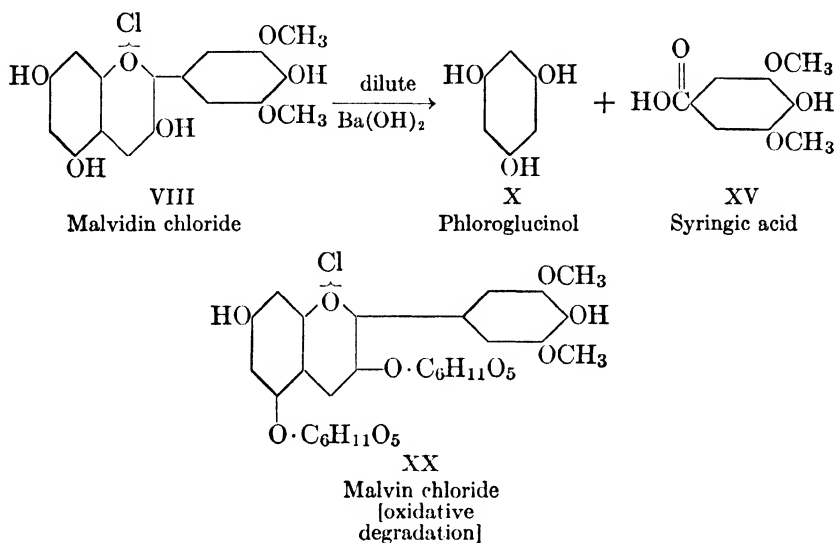
If malvon is XIVa, degradation with dilute sodium hydroxide would yield syringic acid (XV) and a derivative of phenylacetic acid (XVI), 2-glucosido-4,6-dihydroxyphenylacetic acid (not isolated). If XIVb is the correct structure for malvon, syringic acid (XV) would likewise be formed and a derivative of mandelic acid (XVII), 2-glucosido-4,6-dihydroxymandelic acid (not isolated). The structure of syringic acid (XV) was known from previous studies in the tannin group, so the positions of the two methoxyls in the 3',5'-positions of malvin are established. The structure of the two phloroglucinol residues (XVI) and (XVII) is still an open question since neither has been isolated, nor have they been prepared through independent syntheses.

Another mode of attack introduced by Karrer¹⁴ involved the methylation of the anthocyanins themselves, which was followed by a subsequent removal of the sugar group and the identification of the unmethylated position which it originally occupied. It was largely through these approaches that the location of the sugar residue in the monoglycosides was allocated to the 3-hydroxyl position of the anthocyanidin nucleus.

Karrer's work in conjunction with Robinson's synthetical approach, which was being made at about the same time, eventually led to the conclusion that the second sugar residue in the diglucosides occupies position number 5 most generally. The total synthesis of malvin chloride

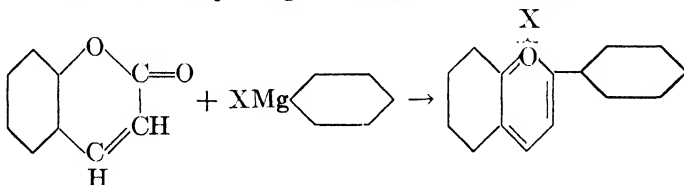
* The corresponding intermediates from other members of the group have not been isolated.

(XX) realized by Robinson represents the crowning achievement of this phase of the anthocyanidin studies (see below).

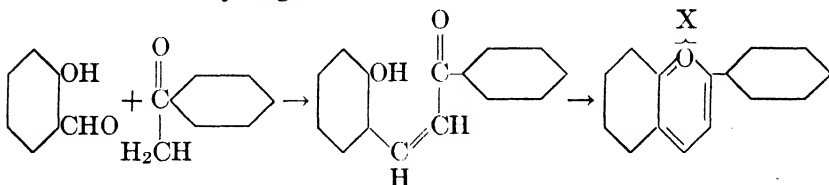


General Methods of Synthesizing the Anthocyanidins and the Anthocyanins. The constitution originally assigned by Willstätter to the three parent types of the class, e.g., pelargonidin (IV), cyanidin (V), and delphinidin (VI), has been confirmed through syntheses carried on independently in the laboratories of Willstätter^{6,7} and Robinson.^{8,10,11,12,13} The resulting synthetic specimens have been carefully compared and identified with the natural products. The two general methods that have been employed are:

1. The addition of aryl Grignard reagents to coumarins:



2. The condensation of *o*-hydroxybenzaldehydes with appropriate ketones followed by ring closure:



Willstätter⁶ used the first of these methods in the synthesis of pelargonidin and cyanidin. Robinson^{10,15} has employed the second method with eminent success in the synthesis of *all* the anthocyanidin types.

The total synthesis of several naturally occurring anthocyanins * by Robinson and his school^{10,15,16} represents an even greater achievement than the synthesis of the six type anthocyanidins. The general procedure of Robinson's method is illustrated by the synthesis of malvin, the 3,5-diglucoside of malvidin, which occurs in the wild mallow and in certain primulas.¹⁶

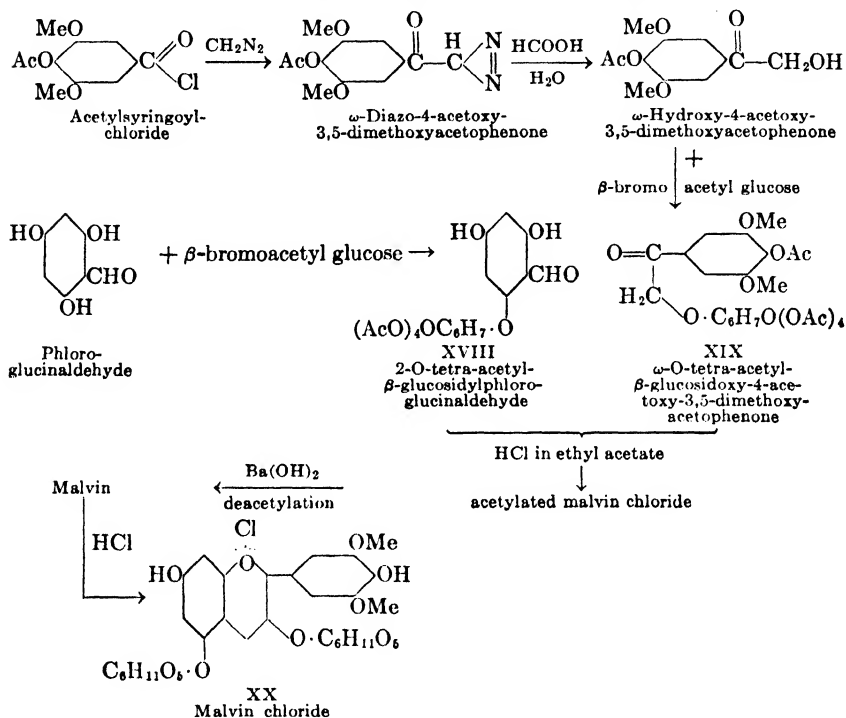
Malvidin is 3,5,7,4'-tetrahydroxy-3',5'-dimethoxy-2-phenylbenzopyrylium chloride (VIII). The synthesis of the diglucoside malvin was accomplished in the following manner. The 2-O-acetylglucoside of phloroglucinaldehyde (XVIII) (2-O-tetra-acetyl- β -glucosidylphloroglucinaldehyde) was condensed with ω -O-tetra-acetyl- β -glycosidoxy-4-acetoxy-3,5-dimethoxyacetophenone (XIX) in dry ethyl acetate solution

¹⁵ Robinson and co-workers, *J. Chem. Soc.*, 2665, 2701 (1931); 2299 (1932).

* These are chrysanthemin, oenin, pelargonin, cyanin, and malvin.

¹⁶ Robinson and co-workers, *ibid.*, 125, 188 (1924); 127, 166 (1925); 1968 (1926).

with hydrogen chloride.* The resulting condensation product was treated with barium hydroxide to remove the acetyl groups from the sugar residues. Then the flavylum salt, malvin chloride, was generated by treatment with hydrochloric acid. No divergences in the properties and behavior of the natural pigment (isolated by Karrer) and the synthetic could be detected. The important reactions involved in this synthesis are presented structurally in the scheme below:



General Properties and Isolation of the Anthocyanins.^{4,5,7,17} As might be expected from the usual occurrence of these pigments in the plant cell sap, all members of the group are soluble in water. They are also quite soluble in the hydroxylic solvents, but they are insoluble in such non-hydroxylic solvents as ether, benzene, or chloroform. Since they cannot be extracted from the plant tissue by means of the volatile solvents, special means of separating the accompanying water-soluble substances had to be developed.

Willstätter^{6,7} recognized at the outset that these pigments are amphoteric substances and that they form true oxonium salts with acids.

* The letter ω (omega) is used to denote a substituent at the end of any chain.

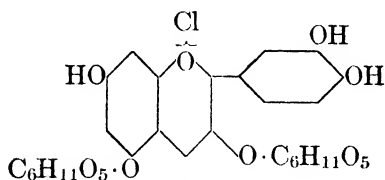
¹⁷ Robinson and Robinson, *Biochem. J.*, **25**, 1687 (1931); **26**, 1647 (1932); **27**, 206 (1933).

These salts are remarkably stable and have extraordinary crystallizing properties. Consequently in the final stages of the isolation, the pigment is usually converted into its hydrochloric or picric acid salt.

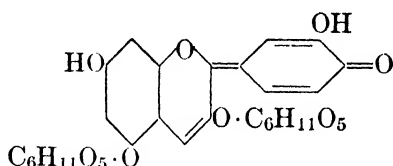
The isolation of a member of this group usually proceeds along the following general lines. The pigment is first extracted from the plant material with methyl or ethyl alcohol containing hydrochloric acid. The crude chloride is then precipitated with ether. It is purified by redissolving in aqueous hydrochloric acid, a suitable quantity of alcohol is added and then ether to effect a reprecipitation of the salt. The final recrystallization may be done with alcoholic hydrochloric acid or aqueous alcoholic hydrochloric acid.

Karrer¹⁸ has recently shown that at least some of the anthocyanins obtained by the above procedure can be purified through the use of the chromatographic adsorption technique of Tswett and that certain pigments in this group heretofore regarded as pure are mixtures containing traces of other anthocyanins.

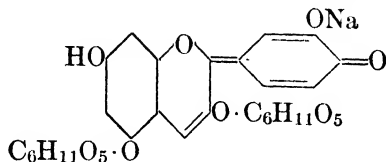
The acid salts of the anthocyanins and anthocyanidins are usually red; the metallic salts with bases are blue. In the neutral state the pigments are purple. Thus, cyanin, the pigment of the blue cornflower and of the rose, is red in solutions of pH 3.0 or less, violet at pH 8.5, and blue at pH 11.0. The red, violet, and blue forms are the oxonium salt (XXI), the color-base* (XXII), and the salt of the color-base (XXIII) (after Willstätter).†



XXI
Oxonium salt of cyanin



XXII
Color base of cyanin



XXIII
Salt of the color base of cyanin

¹⁸ Karrer and co-workers, *Helv. Chim. Acta*, **19**, 28, 1025 (1936).

* As yet no evidence exists in regard to the assumed position of the quinonoid group and the acidic hydroxyl.

† For other methods of formulating the salts and pseudo-bases on the basis of the carbonium and carbenium theory see Hill, *Chem. Rev.* **19**, 27 (1936).

Absorption Spectra of the Anthocyanidins. The anthocyanidins as well as all the anthocyanins studied so far have strong absorption powers over the range of 6,000 to 2,000 Å units. The absorption spectra (p. 1771) of the sugar-free pigments and the glycosides of the pigments are approximately the same. A maximum absorption, the cause of the color, lies in the visible spectrum. All members examined also have a band that lies in the vicinity of 2700 Å. The absorption spectra of the chlorides of three anthocyanidins (concentrations 0.0001–0.00004 molar in ethyl alcohol) are presented in Fig. 1. (After Schou.¹⁹)

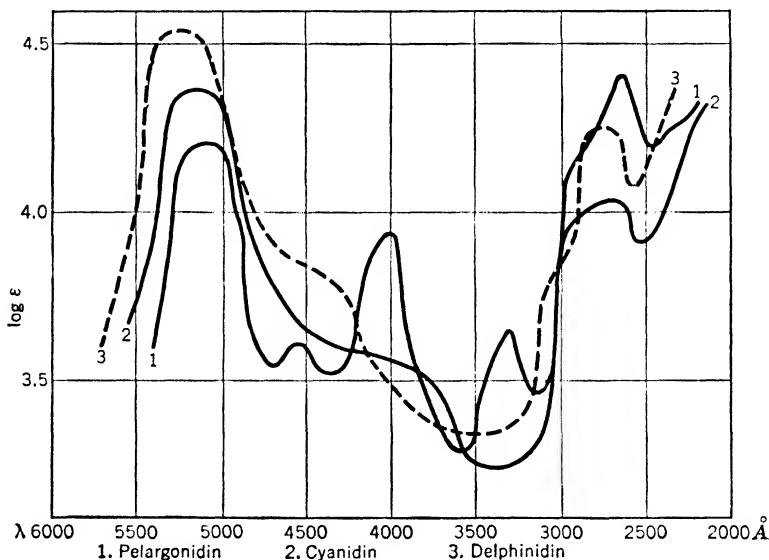


Fig. 1

Factors Affecting the Colors of Anthocyanin Pigments in the Plant.

Recent studies by the Robinsons^{13,17} lead to the belief that the main factors affecting the colors of the anthocyanin pigments in the cell sap are (1) the nature and concentration of the anthocyanins and other colored substances present; (2) the state of aggregation of the anthocyanin in solution, which is determined in part by the pH of the cell sap and the presence or absence of protective colloids of the polysaccharide group (the pentosans); and (3) the presence or the absence of co-pigments (the tannin and flavone glycosides) and possibly also the effect of alkaloids (p. 1018), of traces of iron and other metals that form complex combinations.

The Occurrence of the Anthocyanins.^{4,5,17} Over twenty glycosidic combinations of the various anthocyanidins have been isolated from the

¹⁹ Schou, *Helv. Chim. Acta*, **10**, 907 (1927).

flowers and fruits of plants. The anthocyanins usually occur as mixtures, and the amount in the various flowers varies over a wide range. Thus, the cyanin of the blue cornflower represents 0.75 per cent of the weight of the dry petals. In certain deep red dahlias this pigment comprises over 20 per cent of the dry weight of the petals, and in the dark blue pansy the anthocyanin content (violanin) is approximately 33.0 per cent.

The Robinsons¹⁷ have made an extensive survey on the occurrence of anthocyanins and have developed means of detecting qualitatively the type of anthocyanidin derivative that an extract of the plant tissue in question might contain. In some instances they have been able to distinguish the nature of the sugar residues (e.g., methylpentose or aldopentose) (p. 1450) attached to the pigment.

The methods have been developed from an exhaustive study of the chemical behavior of the pure anthocyanins and anthocyanidins isolated either from natural sources or prepared synthetically. The basis for the methods are the characteristic color reactions given by the anthocyanins with alkalis and ferric chloride and the distribution coefficient of the anthocyanin between immiscible solvents. The tests employed are:

1. Oxidation test. The addition of 10 per cent aqueous sodium hydroxide to a dilute solution of the pigment which is then shaken in the presence of air. Petunidin and delphinidin are destroyed at once; the other members of the group are relatively stable.

2. Extraction with amyl alcohol, addition of sodium acetate and a trace of ferric chloride. Characteristic color reactions are observed. The color is particularly pronounced if cyanidin is present. The violet amyl alcohol solution changes to a pure blue in the last stage of the reaction. Pelargonidin, peonidin, and malvidin do not give the ferric chloride test.

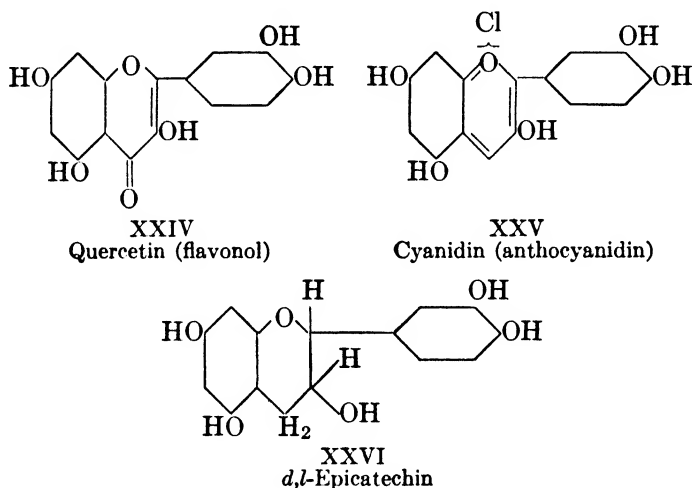
3. Distribution between 1 per cent aqueous hydrochloric acid and a mixture of anisole (5 volumes) and ethyl isoamyl ether (1 volume) containing 5 g. of picric acid in 100 cc. Delphinidin is not extracted by the organic solvent layer; petunidin is taken up to some extent, cyanidin to a considerable extent, whereas malvidin, peonidin, and pelargonidin are completely extracted if the solution is sufficiently dilute.

4. Distribution between 1 per cent hydrochloric acid and a mixture of cyclohexanol (1 volume) and toluene (5 volumes). Delphinidin and petunidin are not extracted, malvidin gives the organic solvent layer a faint blue tint, cyanidin a pale rose tint, while peonidin and especially pelargonidin are extracted to a considerable extent.

The above tests are readily applicable to crude extracts, for usually

only one pigment is involved in the production of the color in the plant material extracted.

The Relationship of the Anthocyanidins to Other Classes of Plant Products.^{6, 12, 13, 20, 21, 22, 23, 24} The anthocyanidins represent a class of substances which from the standpoint of degree of oxidation lie intermediate between the flavonols and the catechins. This is illustrated by a comparison of the anthocyanidin, cyanidin (XXV), to the flavonol, quercetin (XXIV), and to *d,l*-epicatechin (XXVI).



In fact, cyanidin has been obtained *in vitro* from quercetin, by means of reduction with magnesium in aqueous methyl alcoholic hydrochloric acid solution,⁶ and the reduction of cyanidin to *d,l*-epicatechin has also been realized.²⁰ The reverse reaction, that is, the oxidation of the less oxidized substance to a higher stage of oxidation, has so far not been achieved.

The successful conversion of a widely distributed anthoxanthin (quercetin [XXIV]) into a widely distributed anthocyanidin (cyanidin [XXV]) has led to speculations that similar reactions occur in the plant and indicate the course of the phytochemical synthesis. Robinson^{8, 12, 24, 25} is, however, of the opinion that there is little justifi-

²⁰ Freudenberg and co-workers, *Ann.*, **444**, 135 (1925).

²¹ Wheldale, *Nature*, **129**, 601 (1932).

²² Stewart, "Recent Advances in Organic Chemistry," Longmans, Green and Co., London (1930), 6th ed., Vol. II, Chapter VII.

²³ Robinson and Robinson, *Nature*, **130**, 21 (1932).

²⁴ Robinson, *ibid.*, **137**, 172 (1936).

²⁵ Robinson and Robinson, *J. Chem. Soc.*, 744 (1935).

SOME OF THE IMPORTANT PROPERTIES OF THE SIX TYPE ANTHOCYANIDINS

	Pelargonidin	Cyanidin	Delphinidin	Peonidin	Malvidin or Syringidin	Hirsutidin
Color of aqueous solution	Red	Violet red	Bluish red	Violet red	Violet red	Violet red
Solubility of chloride in water	Readily soluble	Only slightly soluble	Very soluble	Readily soluble	Slightly soluble	Slightly soluble
Ferric chloride reaction	Not definite	Intense blue	Intense blue	Faint—not definite	No reaction	No reaction
Behavior toward Fehling's solution	Reduces when warmed	Reduces in the cold	Reduces in the cold	Reduces when boiled	Reduces when boiled	Reduces when boiled
Color change in soda solution	Violet then blue	Violet then blue	Violet then blue	Violet then blue	Violet then greenish blue	Violet then greenish blue
Behavior in aqueous solution	Color fades on standing	Color disappears on heating	Slow fading in the cold; when heated, rapid fading	Color disappears on heating	In very dilute solution color disappears when heated	In very dilute solution color disappears when heated

cation for this view and has suggested that the flavones and anthocyanidins are independently synthesized from a common starting point through a transformation involving an oxidation. Continuing the work of Rosenheim,²⁶ the Robinsons have established the presence in plant tissue of colorless precursors (called leuco-anthocyanidins) which yield colored anthocyanin-like pigments on treatment with hydrochloric acid in the presence of oxygen. The leuco-anthocyanidins have in some instances been obtained in a crystalline form and have been shown to be both glycosides and sugar-free substances.²⁵ They are classified on the basis of solubility in water and the property of being extracted from aqueous solutions with ethyl acetate.

A detailed study of the mechanism of the biosynthesis of the flavonols, anthocyanidins, and catechins will without doubt lead to many interesting results which should be of great significance to our understanding of phytochemical processes in general.*

THE DISTRIBUTION AND OCCURRENCE OF SOME REPRESENTATIVE MEMBERS OF THE SIX TYPE ANTHOCYANIDINS

1. *Pelargonidin derivatives*

Pelargonin	Diglucoside	Scarlet pelargonium, orange-red dahlia, red cornflower
Punicin	Diglucoside (seemingly identical with pelargonin)	<i>Punica granatum</i>
Monardaerin or salvianin	Diglucoside—contains also <i>p</i> -hydroxycinnamic acid and malonic acid	<i>Monarda didyma</i> and <i>Salvia splendens</i> , <i>Selle</i> and <i>coccinea</i> L.
Callistephin	Monoglucoside	<i>Callistephus chinensis</i> , Nees, syn. <i>Aster chinensis</i> L.

2. *Cyanin Derivatives*

Cyanin	Diglucoside	Red rose, blue cornflower, deep-red dahlia
Mekocyanin	Diglucoside	Dark red Mohn (<i>Papaver Rhoeas</i> L.)
Keracyanin	Rhamnoglucoside	Black (dark) cherries
Sambucin	Monoglucoside (apparently identical with chrysanthemin)	Elderberries (<i>Sambucus nigra</i>)
Idaein	Galactoside	Cranberries (mountain)
Chrysanthemin or asterin	Monoglucoside	Scarlet-red winter aster

²⁶ Rosenheim, *Biochem. J.*, **14**, 73 (1920).

* For details on the biological significance of the anthocyanins see references 4, 22, 24, and 25. The distribution of the individual pigments among the flowering plants is treated in references 3, 4, 5, and 17.

3. *Delphinidin Derivatives*

Delphinin	Diglucoside	<i>Delphinium Consolida</i> L.
Violanin	Rhamnoglucoside	<i>Viola trichlor</i> L.
Gentianin	Monoglucoside, contains <i>p</i> -hydroxycinnamic acid	<i>Gentiana acaulis</i> , <i>Gentiana vul-</i> <i>garis</i>

4. *Peonidin Derivatives*

Peonin	Diglucoside	Red peony
Oxycoccicyanin	Monoglucoside	Fruit of <i>Oxycoccus macrocarpus</i> Pers.

5. *Malvidin (Syringidin) Derivatives*

Malvin	Diglucoside	Wild Malve, <i>Primula viscosa</i>
Oenin	Monoglucoside	Blue grape

6. *Hirsutidin Derivatives*

Hirsutin	Diglucoside	<i>Primula hirsuta</i>
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THE FLAVONES

Introduction. The flavones (from the Latin for yellow) represent an important group of pigments that occur in the plant kingdom.^{1,2,3,27,28,29} Of all the natural pigments that can be used as dyestuffs they are by far the most widely distributed in nature.* They occur naturally in combination with rhamnose or glucose as glycosides, sometimes uncombined, and frequently also associated with tannins. One of the members of this group, luteolin, the main coloring matter of the herbaceous plant known as weld (*Reseda luteola*), is said to be the oldest European dyestuff known.^{1,3} Some of the flavone dyestuffs that are still significant economically are weld, young and old fustic, and quercitron bark. The use of these, however, is largely confined to the uncivilized or semi-civilized countries in which they abound. The chemistry of the flavones, which bears a striking resemblance to the anthocyanidin group (p. 1114), was elucidated largely through the researches of von Kostanecki, Herzig, and A. G. Perkin, and dates from the period of 1895 onward.

The basic unit of the flavones is γ -pyrone (I), the anhydride of an unsaturated 1,5-dihydroxy-3-ketone. γ -Pyrone is a colorless solid and

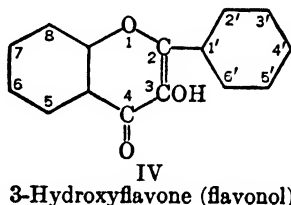
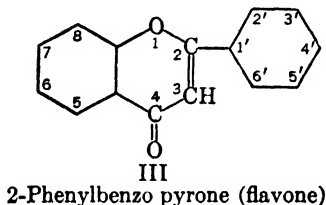
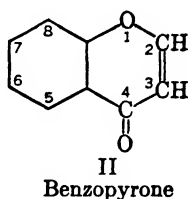
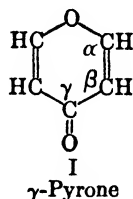
²⁷ Klein (editor), "Handbuch der Pflanzenanalyse," Springer, Vienna (1932), Vol. III, pp. 851-941.

²⁸ Abderhalden, "Biochemisches Handlexikon," Springer, Berlin (1911), Vol. VI.

²⁹ Bömer, Juckenack, and Tillmans, "Handbuch der Lebensmittel Chemie," Springer, Berlin (1933), Vol. I, p. 604.

* That their distribution in plants is practically universal can be readily demonstrated by the color reaction with alkalis. This reaction is best shown by colorless parts of plants, such as white flowers. Placed in ammonia vapor, almost any white flower will turn bright yellow.

has been prepared synthetically by Claisen.³⁰ The simplest aromatic derivative of γ -pyrone is benzopyrone (II), commonly called chromone. Substitution of a benzene residue in position 2 of the γ -pyrone nucleus produces 2-phenylbenzopyrone (III), or flavone. When the hydrogen on carbon atom 3 in the γ -pyrone ring of flavone is substituted by hydroxyl, 3-hydroxyflavone (IV), or flavonol, is formed.



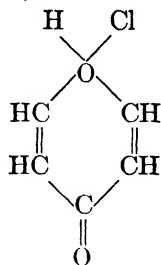
The various flavones and 3-hydroxyflavones, or flavonols, differ from III and IV, respectively, in that substitution of hydrogen atoms by hydroxyl groups has taken place in either the phenyl or benzo radical of the parent formulas. The accompanying table lists a few of the typical members of the group and illustrates the comparative constitution.* The structure of the members listed here has been verified through degradation studies and by syntheses.

Properties of the Flavones. Most of the flavones are yellow crystalline solids (with high melting points), soluble in water, alcohol, dilute mineral acids, and alkalis. From their solutions they may be precipitated by lead acetate, the precipitate being yellow, orange, or red. With ferric chloride a dull green or sometimes a red brown coloration results. The solubility of the flavones in acids is due to the basic properties of the oxygen atom in the γ -pyrone nucleus. The oxygen atom by becoming tetravalent can form additive compounds with acids producing oxonium salts. The salts are, as a rule, more highly colored than the bases from which they are derived, and are generally very unstable in

³⁰ Claisen, *Ber.*, **24**, 118 (1891).

* For a detailed compilation of the structure, physical properties, mode and place of occurrence of the many flavone pigments that have been studied to date see references 1, 2, 3, 27, 28, 29, 30.

the presence of water. The flavones differ in this respect from the anthocyanidins, which yield stable oxonium salts and frequently occur as such in the plant * (p. 1115).



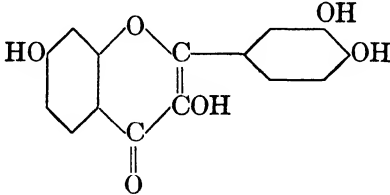
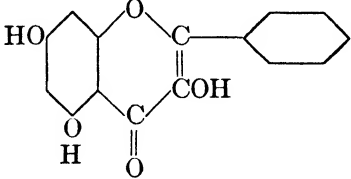
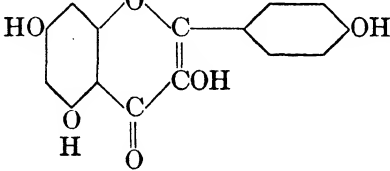
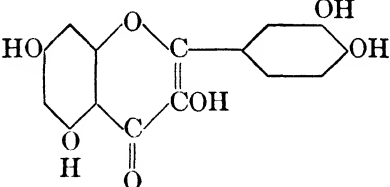
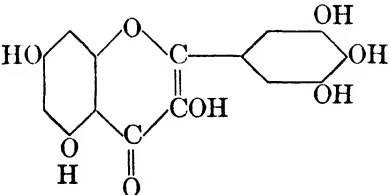
γ -Pyrone·HCl

REPRESENTATIVE FLAVONE PIGMENTS

Name	Structural Formula	Occurrence
Flavone $C_{15}H_{10}O_2$ [2-phenylbenzo- pyrone]		As dust on flowers, leaves, and seeds of various primulas
Chrysin $C_{15}H_{10}O_4$ [5,7-dihydroxy- flavone]		In buds of several varieties of poplar (<i>P. nigra</i> , <i>P. pyramid- alis</i>)
Apigenin $C_{15}H_{10}O_5$ [5,7,4'-tri- hydroxyflavone]		In parsley as glycoside apiin, in yellow dahlias
Luteolin $C_{15}H_{10}O_6$ [5,7,3',4'-tetra- hydroxyflavone]		In weld (<i>Reseda luteola</i>), dyers' broom (<i>Genista tinctoria</i>)

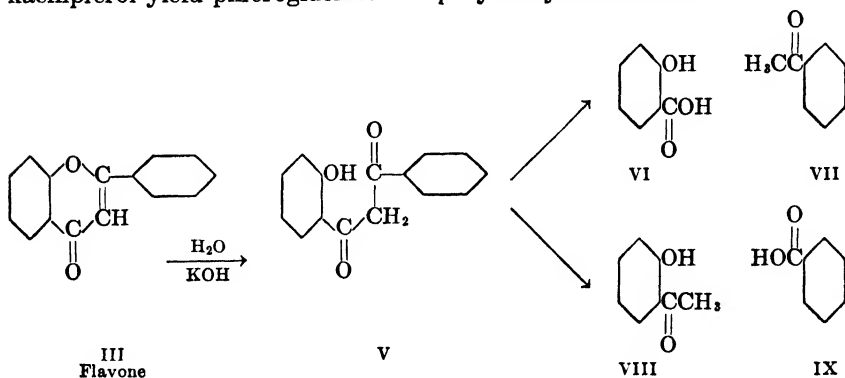
* For details on the biological significance of the flavones, see reference 4; also Haas and Hill, "An Introduction to the Chemistry of Plant Products," 4th ed., Longmans, Green and Co., London (1928), Vol. I.

REPRESENTATIVE FLAVONE PIGMENTS—Continued

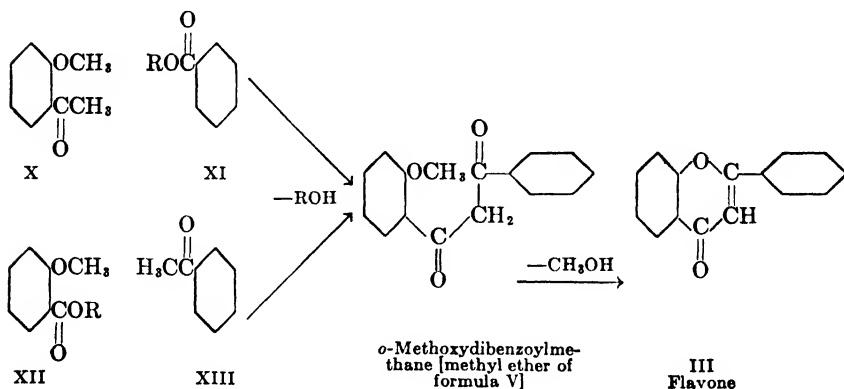
Name	Structural Formula	Occurrence
Fisetin $C_{15}H_{10}O_6$ [3,7,3',4'-tetra- hydroxyflavone]		In wood of young fustic (<i>Rhus</i> <i>cotinus</i> and <i>Quebracho</i> <i>colorado</i>)
Galangin $C_{15}H_{10}O_5$ (flavonol of chrysin) [3,5,7-tri- hydroxyflavone]		In galanga root, the rhizome of <i>Alpinia officinarum</i>
Kaempferol $C_{15}H_{10}O_6$ (flavonol of Apigenin) [3,5,7,4'-tetra- hydroxyflavone]		In blue del- phinium flowers
Quercetin $C_{15}H_{10}O_7$ (flavonol of Luteolin) [3,5,7,3',4'-penta- hydroxyflavone]		As 3-rhamnoside in bark of Ameri- can oak (<i>Quercus</i> <i>tinctoria</i>), leaves of horse chestnut, colored onion scales, etc.
Myricetin $C_{15}H_{10}O_8$ [3,5,7,3',4',5'- hexahydroxy- flavone]		As glycoside in an evergreen native to the Orient, the Myri- caceae family

Degradation of the Flavones. On boiling with alkali the heterocyclic ring system is opened. The course of the degradation can be illustrated with flavone, which forms *o*-hydroxydibenzoylmethane (V). This then degrades in part to salicylic acid (VI) and acetophenone (VII), and in part to *o*-hydroxyacetophenone (VIII) and benzoic acid (IX). On fusion with caustic alkali the flavones are degraded to a phenol and

an acid. Phloroglucinol and protocatechuic acid are commonly formed, and sometimes resorcinol and resorcylic or hydroxybenzoic acids, depending on the substitution in the benzene rings in positions 2, and 5,6 of the γ -pyrone nucleus (I). Thus, quercetin and luteolin yield phloroglucinol and protocatechuic acid (3,4-dihydroxybenzoic acid). Apigenin and kaempferol yield phloroglucinol and *p*-hydroxybenzoic acid.



Synthesis of the Flavones. Various methods have been developed by von Kostanecki, Perkin, Robinson, and others.^{3,2,31,32} One of the most useful general methods involves a condensation of appropriate alkylated *o*-hydroxyacetophenones (X) with esters of aromatic acids, (XI), or esters of alkylated salicylic acid (XII) with acetophenones (XIII) in the presence of sodium.

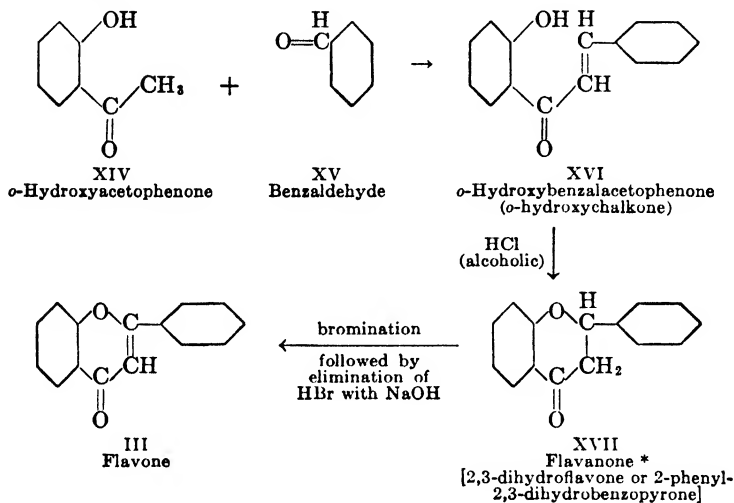


Another general method employs a condensation of *o*-hydroxyacetophenone (or its methoxyl derivative) with benzaldehyde (its hydroxyl

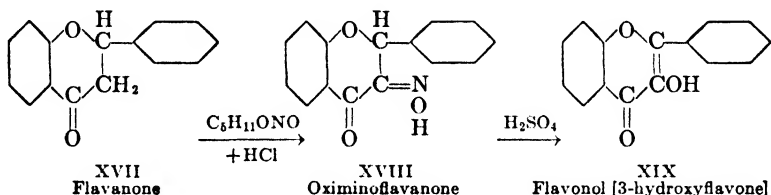
³¹ Hepworth, "Chemical Synthesis," Blackie and Son, London (1924).

³² Abderhalden, "Handbuch der biologischen Arbeitsmethoden," Urban and Schwarzenberg, Berlin (1922), Vol. I, Part 10, Supplement 84.

or methoxyl derivatives). The appropriate hydroxyl or methoxyl derivatives of the two starting products are chosen depending on the flavone sought.



When flavanone † (XVII) is treated with amyl nitrite and strong hydrochloric acid in alcoholic solution, the oximino (isonitroso) compound (XVIII) is formed, which on boiling in acetic acid solution with 10 per cent sulfuric acid forms 3-hydroxyflavone or flavonol (XIX).



Finally, flavonol derivatives can be obtained by reacting the appropriate ω -methoxyacetophenone with appropriate phenolic acid anhydrides. An example of this reaction which was developed by Robinson is presented below.³³

The synthesis of quercetin (XXVI) (3,5,7,3',4'-pentahydroxyflavone) was realized by von Kostanecki³⁴ in 1904. Phloroacetophenone-4,6-di-

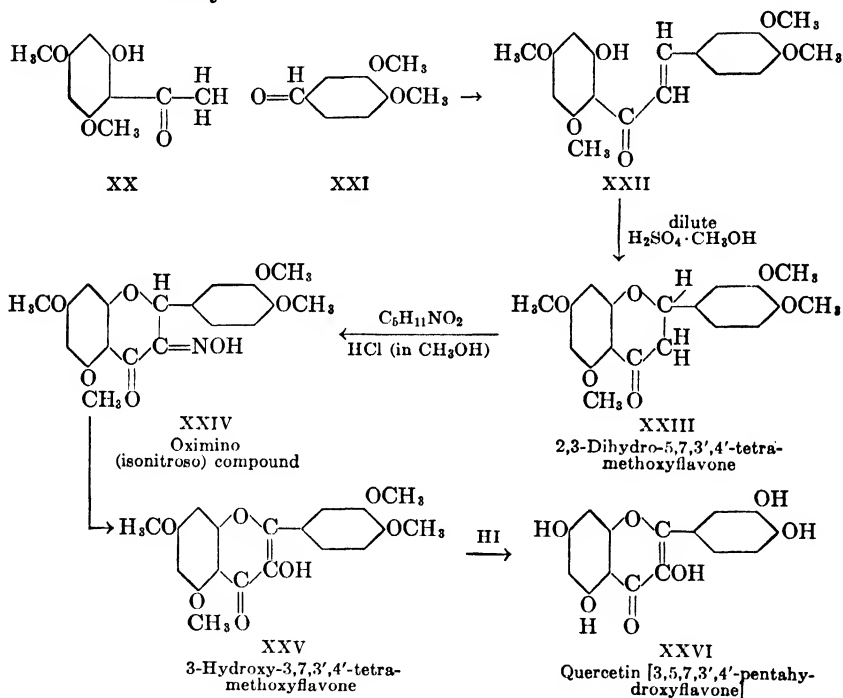
* When the carbon 3 in the flavone is completely reduced with the elimination of the double bond the structure is called a *flavanone*.

† Derivatives of flavanone also occur in nature. Hesperitin, the glycoside occurring in oranges, is a flavanone. It is 5,7,3'-trihydroxy-4'-methoxyflavanone-7-rhamnoside. See references 1, 2, 3, 27, 28, 29.

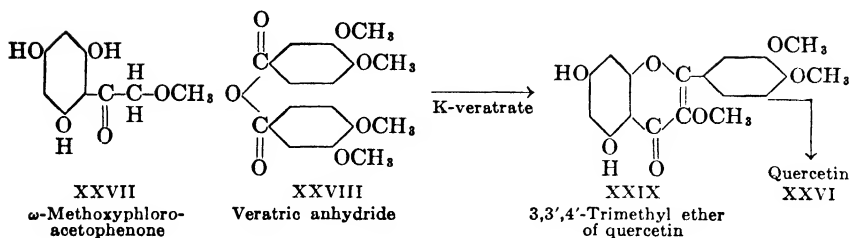
³³ Allan and Robinson, *J. Chem. Soc.*, **125**, 2192 (1924); 2334 (1926).

³⁴ von Kostanecki, Lampe, and Tambor, *Ber.*, **37**, 1402 (1904).

methyl ether (XX) was condensed with veratric aldehyde (XXI). The resulting 2-hydroxy-4,6,3',4'-tetramethoxychalcone (XXII) was then boiled with dilute sulfuric acid in methyl alcohol and converted into the 2,3-dihydrotetramethoxyflavone (XXIII). The introduction of the hydroxyl on carbon number 3 in the pyrone ring was done in the usual manner through the oximino compound (XXIV). Finally, removal of the four methoxyl residues from tetramethoxyquercetin (XXV) produced quercetin (XXVI), identical in all respects with the naturally occurring pigment. The various stages of this synthesis can be illustrated structurally as follows:



A direct synthesis of quercetin (XXVI) was realized in 1926 by Robinson.³³ ω -Methoxyphloroacetophenone (XXVII) was condensed with the anhydride of 3,4-dimethoxybenzoic acid (veratric anhydride) (XXVIII) in the presence of the potassium salt of veratric acid. A molecule of veratric acid is regenerated as a result of the condensation which produces the 3,3',4'-trimethyl ether of quercetin (XXIX). Removal of the methoxyl residues from the trimethoxyquercetin (XXIX) yielded the free pigment (XXVI), m.p. 313–314°, identical in all respects with the naturally occurring product.



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CHAPTER 14

CAROTENOIDS

THE POLYENE PIGMENTS OF PLANTS AND ANIMALS

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INTRODUCTORY

Introductory and Historical. The name "carotenoid" is applied to a class of polyene pigments which are found widely distributed in both the vegetable and the animal kingdom, and includes most, or at least a large majority, of those naturally occurring pigments heretofore known as luteins, lipochromes, lipoxanthins, or chromolipoids.

The one first discovered, most plentiful in nature, and best known, is carotene itself, which was isolated by Wackenroder,¹ in 1831, in ruby-red crystals, from the root of the cultivated carrot (*Daucus carota*), and named by him "carotin." It is a mixture of at least three isomers, *alpha*-, *beta*-, and *gamma*-carotenes, the *beta*- being the dominant form. Since all three are polyene hydrocarbons, of the formula $C_{40}H_{56}$, the original spelling has been changed to "carotene," in conformity with present international rules for the nomenclature of organic compounds.

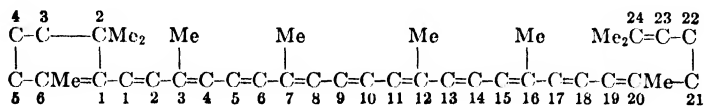
As a class, the carotenoids are of exceptional interest, not only because of their widespread occurrence in nature, their chemical and biological connections or associations with the terpenes, sterols, lipids, albumins, and other important products of the living cell, and the recognition of several of them as progenitors of vitamin A, but also for the reason that a wholly new and fascinating domain has been opened by their study, a domain whose further exploration holds brilliant promise of discoveries of importance to chemistry, biology, and medicine, and of benefit to mankind.

Not All Yellow or Orange Natural Pigments Are Carotenoids. The carotenoids are not the only yellow or orange pigments found in plants

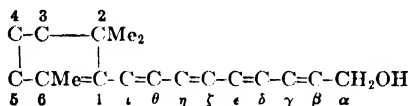
¹ Wackenroder, *Geigers Mag. Pharm.*, **33**, 144 (1831).

⁵ Kuhn and Brockmann, *Ann.*, **516**, 95 (1935).

Heilbron and his co-workers use this Kuhn and Brockmann system, and give the following numbering for the carbon atoms in *gamma*-carotene:⁶



and for vitamin A,⁷



⁶ Heilbron and Lythgoe, *J. Chem. Soc.*, 1376 (1936).

⁷ Davies, Heilbron, Jones, and Lowe, *ibid.*, 584 (1935).

TABLE I
NATURALLY OCCURRING SIMPLE CAROTENOIDS

Name	Molecular Formula	Occurrence	Appearance	M. P. (corr.)	Optical Activity	Absorption Max. (CS ₂) m μ	Substituents	Double Bonds	Cycles	Remarks	References
Antheraxanthin	C ₄₀ H ₅₆ O ₃ or C ₄₀ H ₅₈ O ₃	Tiger lily (<i>Lilium tigrinum</i>)	Bright-yellow leaflets (alc.)	211°		512.5 481 448				In anthers, as ester. Abs. max. + SbCl ₃ (CHCl ₃) = 587 m μ . Resembles zeaxanthin	8 9
Astacin	C ₄₀ H ₄₈ O ₄	Lobster shells (<i>Astacus gammarus</i>)	Violet needles of metallic luster (pyridine + H ₂ O)	240-243° 265-267°		500	(CO) ₄	13* 11	2	As ester, or chromoprotid	10 11
Astetric acid	C ₂₈ H ₄₀ O ₄ or C ₄₀ H ₆₀ O ₆	Starfish (<i>Asterias rubens</i>)	Violet-black powder	185°			COOH			In dorsal skin, as bluish violet chromoprotid	12 13
Azafrin	C ₃₇ H ₃₈ O ₄	<i>Escobedia scabrifolia</i> , etc.	Orange-red prisms	212°	[α] _D ²⁰ Cd = -75.5° (EtOH)	486 457	(OH) ₂ COOH	7	1		5
α -Bacteriopurpurin		<i>Rhodobacillus palustris</i>	Brownish red crystals			585-555 540-515 500-485				Also called α -bacterioerythrin	14

β -Bacteriopurpurin	<i>Rhodospirillum</i>						560-535 520-490 480-460					Also called β -bacterioerythrin	14
α -Bacterioruberin	<i>Bacterium halobium</i>					Violet needles	522 490 466 (EtOH)		OH (?)				14
β -Bacterioruberin	<i>Bacterium halobium</i>					Violet needles	502 482 452 (EtOH)		OH (?)				15
Bixin	Annatto (<i>Bixa orellana</i>)	$C_{31}H_{48}O_4$		198°	$\pm 0^\circ$	Violet needles	523.5 489 457		COOH COOCH ₃	9	0		16 17
Capsanthin	Pepper (<i>Capsicum annuum</i>)	$C_{40}H_{56}O_2$		175-176°	$[\alpha]_D^{25} = +36^\circ$ (CHCl ₃)	Dark carmine-red long needles	543 503.5		(OH) ₂ CO	10		+ (iso-PrO) ₂ Al = Capsanthol, m. p. 175-176°	18 19
Capsorubin	Pepper (<i>Capsicum annuum</i>)	$C_{40}H_{56}O_4$		198°		Violet-red needles (C ₆ H ₆ + benzene)	541 503 468		(OH) ₂ (CO) ₂	9			20
α -Carotene	Carrot (<i>Daucus carota</i>), green leaves, etc.	$C_{40}H_{56}$		187°	$[\alpha]_D^{25} = +380^\circ$ (C ₆ H ₆)	Dark red plates or pointed prisms	509 477			11	2	Refractive index (CHCl ₃) 1.451 Vitamin A activity	21
β -Carotene	Carrot, <i>Urtica urens</i> , green leaves, etc.	$C_{40}H_{56}$		184°	$\pm 0^\circ$	Dark red plates	521 485 450			11	2	Refractive index (CHCl ₃) 1.453 Vitamin A activity	21

TABLE I—Continued
NATURALLY OCCURRING SIMPLE CAROTENOIDS

Name	Molecular Formula	Occurrence	Appearance	M. P. (corr.)	Optical Activity	Absorption Max. (CS ₂) mμ	Substituents	Double Bonds	Cycles	Remarks	References
γ-Carotene	C ₄₀ H ₅₆	<i>Gonocaryum pyri-forme</i> , carrot, etc.	Stout dark red prisms with bluish luster (C ₆ H ₅ + MeOH)	178°	±0°	533.5 496 463		12	1	Vitamin A activity	22
δ-Carotene	C ₄₀ H ₅₆	<i>Gonocaryum pyri-forme</i>	Small needles	172°		526 490 457					23
Citraurin		Orange peel		144–145°		523 488 457	OH (?) CHO			Oxime m. p. 181–182° (corr.); mol. wt. = 376–423	24
Crocin	C ₂₀ H ₁₄ O ₄	Saffron (<i>Crocus sativus</i>)	Scarlet-red leaflets (pyridine)	285°	±0°	482 453	(COOH) ₂	7			25 26 27
Cryptoxanthin	C ₄₀ H ₅₆ O	Ground cherry (<i>Physalis alkekengi</i>)	Reddish violet prisms	169°	±0°	518 483 453	OH	11	2	Vitamin A activity	20 28 29 30
Cynthiaxanthin		<i>Halocynthia papillosa</i>	Yellow prisms	188–190°		517 483 452	OH			Resembles zeaxanthin	31

Echinone	$C_{40}H_{56}O$ $\pm H_2$	Sea urchin (<i>Echinus esculentus</i>)	Violet needles	192–193°		520 488 450				Apparently related to β -carotene. Exhibits vitamin A activity	32
Eschscholtzia carotenol		California poppy (<i>Eschscholtzia californica</i>)	Purplish red needles	180–184°	Active					In flowers as ester	33
Euglenarhodone	$C_{40}H_{56}O_4$	<i>Euglena heliobesens</i>	Violet-brown crystals of metallic luster	227–228°		ca. 550 to 450; Max. 505	(CO) ₄	11			34
Flavorrhodin		Purple bacteria (<i>Rhodospirillum rubrum</i>)				502 472				Apparently a hydrocarbon	35
Flavoxanthin	$C_{40}H_{56}O_3$	Buttercup (<i>Ranunculus acer</i>)	Small golden yellow prisms with bluish luster (MeOH)	184°	$[\alpha]_D^{20} C_6H_6 = +190^\circ$	478 447.5 420	(OH) ₃	11	2		36
Fucoxanthin	$C_{40}H_{56}O_6$	Brown algae (<i>Fucus vesiculosus</i> , and others)	Stout needles (Et ₂ O + Petroleum ether)	160.5°	$\pm 0^\circ$	510 477 445	(OH) ₄ (CO) ₂	10			37
Glycymerin		Sex organs of <i>Pectunculus glycymeris</i> (a mussel)		148–153°		495					38 39

TABLE I—Continued
NATURALLY OCCURRING SIMPLE CAROTENOIDs

Name	Molecular Formula	Occurrence	Appearance	M. P. (corr.)	Optical Activity	Absorption Max. (CS ₂) mμ	Substituents	Double Bonds	Cycles	Remarks	References
Isolutein		Green leaves			±0°						40
Lycopene	C ₄₀ H ₅₆	Red tomato (<i>Lycopersicon esculentum</i>)	Brownish violet needles	175°	±0°	548 507.5 477		13	0		41 42
Lycophyll	C ₄₀ H ₅₆ O ₂	Bitter nightshade (<i>Solanum dulcamara</i>)					(OH) ₂			In the berries	43
Lycoxanthin	C ₄₀ H ₅₆ O	Tomato, and bitter nightshade (berries)		168°			OH				43
Myxoxanthin	C ₄₀ H ₅₄ O	Blue-green algae (<i>Myxophyceae</i>)	Deep copper-colored needles (Et ₂ O + MeOH)	168–169°		488	CO	12	1	+ (iso-PrO) ₂ Al = myxoxanthol	6 44
Myxoxanthophyll	C ₄₀ H ₅₂ O; ± H ₂	Blue-green algae (<i>Myxophyceae</i>)		169–170°	[α] _D = –255° (EtOH)	518 484.5 450 (CHCl ₃)	(OH) ₂ (?)				6

Pectenoxanthin	$C_{40}H_{72}O_2$ or $C_{40}H_{74}O_2$	Sex glands of <i>Pecten maximus</i> (a mussel)	Long brownish red prisms (pyridine + H_2O)	185°		518 488 454	11	2			45 46
Pentaxanthin	$C_{40}H_{74}O_4$ $\pm H_2$	Sea urchin (<i>Echinus esculentus</i>)	Red needles	209-210°		506 474 444					47
Retinene		In eyes of dark-adapted animals							+ $SbCl_3(CHCl_3)$ = abs. band at 666 $m\mu$		48 49 50 51
Rhodopin		Purple bacteria (<i>Rhodospirillum rubrum</i>)	Small clumps of crystals (C_6H_6 + petroleum ether)	ca. 168°		547 508 478	12		(OH) (?)		35 52
Rhodopurpurin	$C_{40}H_{76}$ or $C_{40}H_{78}$	Purple bacteria (<i>Rhodospirillum rubrum</i>)		162°		550 511 479	13				35 52
Rhodovibrin		Purple bacteria (<i>Rhodospirillum rubrum</i>)	Small dark red clumps	168°		556 517			OH		35
Rhodoviolascidin	$C_{42}H_{80}O_2$	Purple bacteria (<i>Rhodospirillum rubrum</i>)	Glistening violet spindle-shaped crystals	218°	$\pm 0^\circ$	573.5 534 496	13	1	(OMe) ₂		35 52 53

TABLE I—Continued
NATURALLY OCCURRING SIMPLE CAROTENOIDS

Name	Molecular Formula	Occurrence	Appearance	M. P. (corr.)	Optical Activity	Absorption Max. (CS ₂) mμ	Substituents	Double Bonds	Cycles	Remarks	References
Rhodoxanthin	C ₄₀ H ₅₆ O ₂	Yew (<i>Taxus baccata</i>)	Blue-black laccolate leaflets	219°	±0°	564 525 491	(CO) ₂	12	2		54
Rubixanthin	C ₄₀ H ₅₆ O	Sweethrier (<i>Rosa rubiginosa</i>)	Lustrous coppery needles (C ₆ H ₅ +Me-OH)	160°	±0°	533 494 461	OH	12	1		55
Salmonic acid		Salmon (<i>Salmo salar</i>)	Dark violet crystals			485 (pyridine)	COOH			From red muscle flesh or eggs	56
Sarcinene		<i>Sarcina lutea</i>				469 440 415 (petroleum ether)					57 58
Solanum dulcamara pigment		Bitter nightshade (<i>Solanum dulcamara</i>)	Violet - red prisms	151°							59
Spirilloxanthin	C ₄₈ H ₆₆ O ₂	Sulfur bacteria (<i>Spirillum rubrum</i>)	Red crystals	218-219°			OH	15		Very sensitive to light	60 61

Sulcataxanthin	$C_{40}H_{52}O_8$ (?)	<i>Anemone sulcata</i> (a sea anemone)				516 482 450						62
Taraxanthin	$C_{40}H_{56}O_4$	Dandelion (<i>Taraxacum officinale</i>)	Brownish yellow prisms of coppery luster	185.5°	$[\alpha]^{25}_{C_6} = +200^\circ$ (AcOEt)	501 469 440	(OH) ₄ (?)	11	2			63 64
Torulene		<i>Torula rubra</i>	Crystals darker than β -carotene	185°		565 522 488						65 66
Violaxanthin	$C_{40}H_{56}O_4$	Yellow pansy (<i>Viola tricolor</i>)	Reddish brown long needles	207°	$[\alpha]^{20}_{C_6} = +35^\circ$ (CHCl ₃)	500.5 469 440	(OH) ₄ (?)	10	2			67
Violerythrin		<i>Actinia equina</i> (a sea anemone)					COOH (?)			Stable only in alkaline solution		62
Xanthophyll (Lutein)	$C_{40}H_{56}O_2$	Green parts of plants	Ruby-red prisms	165°	$[\alpha]_{C_6} = +165^\circ$ (C ₆ H ₆)	508 475 445	(OH) ₂	11	2	Refractive index (CHCl ₃) 1.448		3 68 69
Zeaxanthin	$C_{40}H_{56}O_2$	Yellow corn (<i>Zea mays</i>)	Golden orange leaflets (MeOH)	215.5°	$\pm 0^\circ$	517 482 450	(OH) ₂	11	2			70 71 72

TABLE II
NATURALLY OCCURRING COMPOUND CAROTENOIDS

Name	Molecular Formula	Occurrence	Appearance	M. P. (corr.)	Optical Activity	Absorption max. (CS ₂) $m\mu$	Substituents	Double Bonds	Cycles	Remarks	References
Actinioerythrin		<i>Actinia equina</i> (a sea anemone)	Brownish violet rhombohedra	85°		574 533 495				Ester of violerythrin	39 73
Astacein		Lobster shells (<i>Astacus gammarus</i>)								Probably dipalmitate of 4,4'-dienol form of astacin	74
Crocin	C ₄₄ H ₆₄ O ₁₄	Saffron (<i>Crocus sativus</i>)		186°			(COOH) ₂			Digentiobioside of crocetin	75 76
Helenien	C ₇₂ H ₁₁₀ O ₄	Marigold (<i>Tagetes</i>)	Fine red needles (EtOH)	92°	±0°	511 478 446	(OH) ₂	11	2	Xanthophyll dipalmitate	77
Physalien	C ₇₂ H ₁₁₀ O ₄	Ground cherry (<i>Physalis alkekengi</i>)	Red needles	98.5- 99.5°	±0°	520 484 452	(OH) ₂	11	2	Zeaxanthin dipalmitate	78 79 80
Sarcina pigment		<i>Sarcina lutea</i>				490 460 433				"Chiefly a xanthophyll-like ester"	81

- ⁸ Karrer, *Helv. Chim. Acta*, **19**, E33 (1936).
⁹ Karrer and Oswald, *ibid.*, **18**, 1303 (1935).
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³⁴ Tischer, *Z. physiol. Chem.*, **239**, 257 (1936).
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³⁸ Lederer, *Comp. rend. soc. biol.*, **113**, 1015 (1933).
³⁹ Lederer, *ibid.*, **113**, 1391 (1933).
⁴⁰ Strain, *Science*, **83**, 241 (1936).
⁴¹ Karrer *et al.*, *Helv. Chim. Acta*, **14**, 435 (1931).
⁴² Kuhn and Grundmann, *Ber.*, **65**, 1880 (1932).
⁴³ Zechmeister and v. Cholnoky, *Ber.*, **69**, 422 (1936).
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⁵⁹ Zechmeister and v. Cholnoky, *Naturwissenschaften*, **23**, 407 (1935).
⁶⁰ van Niel and Smith, *Arch. Mikrobiol.*, **6**, 219 (1935).

- ⁶¹ van Niel, "Ann. Rept., Div. of Plant Biol., Carnegie Inst. Washington," 180 (1933-1934).
- ⁶² Heilbron, Jackson, and Jones, *Biochem. J.*, **29**, 1384 (1935).
- ⁶³ Kuhn and Lederer, *Z. physiol. Chem.*, **200**, 108 (1931).
- ⁶⁴ Karrer and Morf, *Helv. Chim. Acta*, **15**, 863 (1932).
- ⁶⁵ Fink and Zenger, *Wochschr. Brau.*, **51**, 89 (1934).
- ⁶⁶ Lederer, *Compt. rend.*, **197**, 1694 (1933).
- ⁶⁷ Karrer and Solmssen, *Helv. Chim. Acta*, **19**, 1024 (1936).
- ⁶⁸ Nilsson and Karrer, *ibid.*, **14**, 843 (1931).
- ⁶⁹ Brockmann and Völker, *Z. physiol. Chem.*, **224**, 193 (1934).
- ⁷⁰ Karrer *et al.*, *Helv. Chim. Acta*, **13**, 268 (1930).
- ⁷¹ Karrer, Morf, v. Krauss, and Zubrys, *ibid.*, **15**, 490 (1932).
- ⁷² Kuhn and Grundmann, *Ber.*, **67**, 596 (1934).
- ⁷³ Fabre and Lederer, *Bull. soc. chim. biol.*, **16**, 105 (1934).
- ⁷⁴ Karrer, Loewe, and Hübner, *Helv. Chim. Acta*, **18**, 96 (1935).
- ⁷⁵ Karrer and Miki, *ibid.*, **12**, 985 (1929).
- ⁷⁶ Karrer and Salomon, *ibid.*, **16**, 643 (1933).
- ⁷⁷ Kuhn and Winterstein, *Naturwissenschaften*, **18**, 754 (1930).
- ⁷⁸ Kuhn and Wiegand, *Helv. Chim. Acta*, **12**, 499 (1929).
- ⁷⁹ Kuhn *et al.*, *Ber.*, **63**, 1489 (1930).
- ⁸⁰ Kuhn *et al.*, *Z. physiol. Chem.*, **206**, 41 (1932).
- ⁸¹ Nakamura, *Bull. Chem. Soc. Japan*, **11**, 176 (1936).

TABLE III

NATURALLY OCCURRING CAROTENOIDS OF UNDETERMINED CONSTITUTION

Acanthodiaptomus yamanacensis, Brehm, contains a carotenoid pigment, either free or as an ester.⁸²

Bacillus lombardo pellegrini. Chargaff and Lederer⁸³ report the presence therein of a carotenol, with absorption maxima at 491 and 458 m μ .

Coralin is the name given to a carotenoid, isolated by Reader,⁸⁴ from *Streptothrix corallinus*. Its ether solution showed absorption bands at 509-485, and 465-455 m μ .

Grassberger bacillus produces a carotenol, whose absorption bands show maxima at 506 and 477 m μ .⁸⁵

Green leaves, according to Strain,⁴⁰ in addition to known carotenoids, contain the following new carotenols:

1. Absorption maxima at 451 and 422 m μ in alcohol; $[\alpha]_{D}^{18} = -56^{\circ}$, in CHCl₃.
2. Absorption maxima at 446.7 and 437.4 m μ in EtOH.
3. Several others, in small amounts.

New carotenoids recently announced are: *eloxanthin*, from *Elodea canadensis*;^{84c} *leptotene*, from acid-fast lepra bacteria;^{84b} *lutein isomer*, from *Ulex europaeus*.^{84c}

Chemical Classification and Structural Formulas

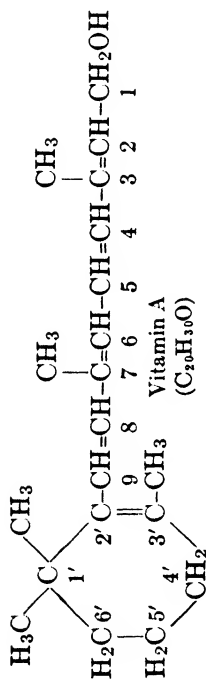
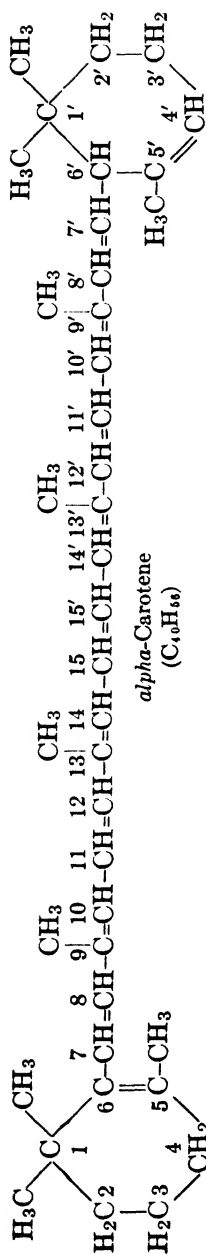
Carotenoids may be divided, on the basis of their elementary composition, into the hydrocarbons (lycopene and the carotenes) and the oxygenated compounds (xanthophylls, bixin, etc.). Of these two groups,

⁸² Suginome, Ueno, and Watanabe, *J. Chem. Soc. Japan*, **56**, 1199 (1935).

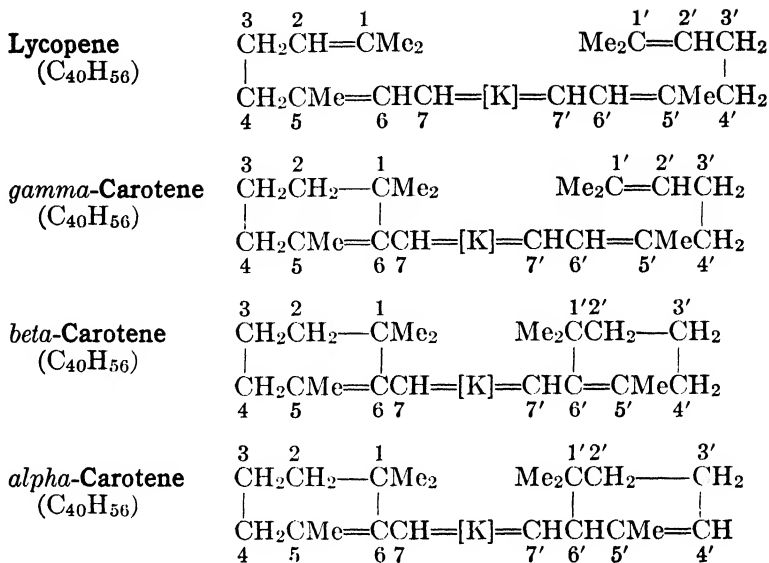
⁸³ Chargaff and Lederer, *Ann. inst. Pasteur*, **54**, 383 (1935).

⁸⁴ Reader, *Biochem. J.*, **19**, 1039 (1925).

⁸⁴ (a) Hey, *Biochem. J.*, **31**, 532 (1937). (b) Grundmann and Takeda, *Naturwissenschaften*, **25**, 27 (1937). (c) Schön, *Biochem. J.*, **30**, 1960 (1936).



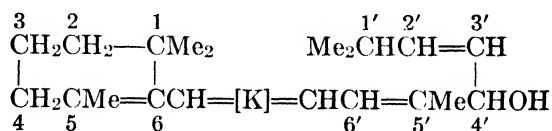
The formulas then appear as follows:



Alcohols (Carotenols)

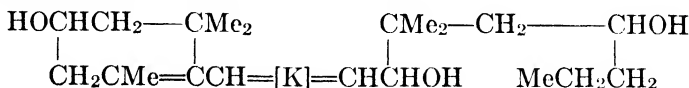
- Lycoxanthin** (C₄₀H₅₆O) = 3-Hydroxylycopene.
Lycophyll (C₄₀H₅₆O₂) = 3,3'-Dihydroxylycopene.
Rubixanthin (C₄₀H₅₆O) = 3-Hydroxy-*gamma*-carotene.
Cryptoxanthin (C₄₀H₅₆O) = 3-Hydroxy-*beta*-carotene.
Zeaxanthin (C₄₀H₅₆O₂) = 3,3'-Dihydroxy-*beta*-carotene.
Xanthophyll (C₄₀H₅₆O₂) = 3,3'-Dihydroxy-*alpha*-carotene.
Flavoxanthin (C₄₀H₅₆O₃) = C₄₀H₅₃(OH)₃.
Taraxanthin (C₄₀H₅₆O₄) = C₄₀H₅₂(OH)₄ (?)
Violaxanthin (C₄₀H₅₆O₄) = C₄₀H₅₂(OH)₄ (?)

Myxoxanthol (C₄₀H₅₆O), which results from the action of aluminum isopropoxide on myxoxanthin, has been assigned the following formula by Heilbron and Lythgoe:⁶



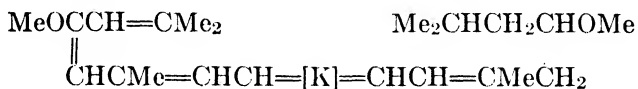
It will be noted that this carbon skeleton differs from that of *gamma*-carotene in having a double bond between carbons 2' and 3', instead of between 1' and 2'.

Capsanthol ($C_{40}H_{60}O_3$), which has been obtained by the reduction of capsanthin by aluminum isopropoxide, has been assigned the following structure by Karrer and Hübner^{8,18}



Ethers

Rhodoviolascin ($C_{42}H_{60}O_2$) = $C_{40}H_{54}(OCH_3)_2$

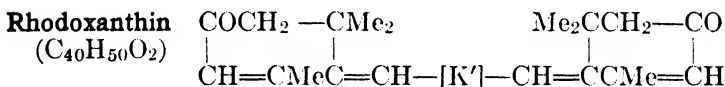
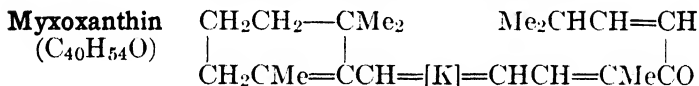


Esters of Carotenols

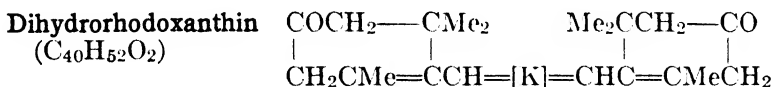
Helenien ($C_{72}H_{116}O_4$) = Xanthophyll dipalmitate.

Physalien ($C_{72}H_{116}O_4$) = Zeaxanthin dipalmitate.

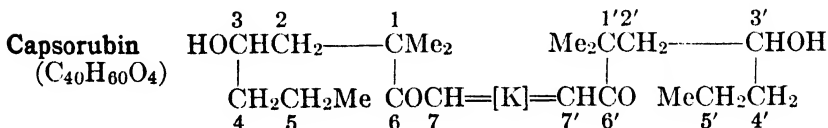
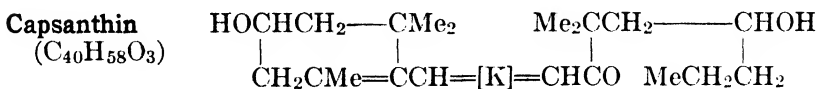
Ketones and Hydroxyketones



By reduction with zinc dust and acetic acid, or catalytically, it yields



By the action of aluminum isopropoxide, this can be reduced to zeaxanthin.



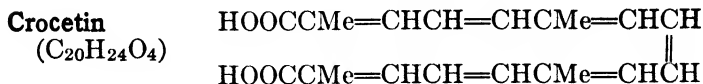
Fucoxanthin ($C_{40}H_{60}O_6$) = 5,5'-Dihydroxycapsorubin (?).

Reduction of the keto groups, followed by cyclodehydration, has been suggested as a possible explanation of the fact that in the living *Fucus vesiculosus*, fucoxanthin is found but no zeaxanthin, whereas in the dead alga the reverse is true.

Euglenarhodone ($C_{40}H_{48}O_4$) = 2,4,2',4'-Tetraketo-*beta*-carotene.

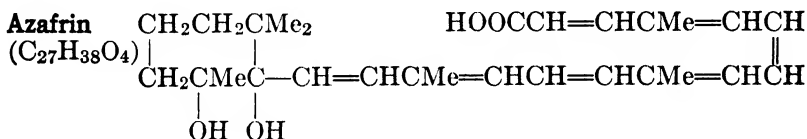
Astacin ($C_{40}H_{48}O_4$) = 3,4,3',4'-Tetraketo-*beta*-carotene.

Acids and Derivatives



Crocin ($C_{44}H_{64}O_{24}$) = Crocetin digentiobioside.

Bixin ($C_{25}H_{30}O_4$) $\text{HOCCCH}=[K]=\text{CHCOOMe}$.



Asteric acid = $C_{28}H_{40}O_4$ or $C_{40}H_{60}O_6$ (?)

OCCURRENCE

Phytocarotenoids. The phytocarotenoids are found in the lower as well as in the higher phyla, from the Thallophyta to and including the Spermatophyta, and may be present in almost any part of the plant. They rarely occur singly, but several are usually found in close association in the same locale. For example, at least five such pigments coexist in the purple bacteria (*Rhodovibrio*).

With few exceptions, they are insoluble in water and hence not present in the cell fluids, differing in this respect from the flavones and anthocyanins (p. 1138), for example. One of the few water-soluble carotenoids is crocin, the chief constituent of the saffron pigment, which is a gentiobioside of the carotenoid, crocetin. Combination with glycoses of albumins is one of the ways by which the living cell converts insoluble into soluble products.

The carotenoids which have been investigated most thoroughly are mainly those of the Spermatophyta and those of certain algae. In general, the fungal-, bacterial-, and zoöcarotenoids have been much less studied. Hence our knowledge of them is still meager. Most bacteria of orange color have been found to contain carotene.⁸⁹

⁸⁹ Ingraham, Fred, and Steenbock, Wis. Agr. Expt. Sta., *Bull.*, **430** (*Ann. Rept.* 1933-4), 104 (1935).

Zoöcarotenoids. Of the phytocarotenoids which occur in animals, lycopene, the carotenes, and carotenols (free or combined) are frequently encountered. Those which appear to be peculiar to animals are astacin, asteric acid, cynthiaxanthin, echinenone, glycymerin, pectenoxanthin, pentaxanthin, retinene, salmic acid, sulcataxanthin, and violerythrin.

Vertebrates, as a rule, appear unable to synthesize carotenoids, and those which they contain, with but few exceptions, are phytocarotenoids taken in with their food. In the body of the water frog (*Rana esculenta*), *beta*-carotene, xanthophyll, and zeaxanthin have been found. As this frog lives chiefly on animal food, it would appear that phytocarotenoids can pass unchanged through several different animal organisms.⁹⁰

The source of the astacin found in a mixed salmon oil and in the liver oil of *Cyclopterus lumpus* is probably the crustaceans on which these fish feed.⁹¹

Invertebrates, on the other hand, do seem to possess the power either to synthesize these polyene pigments for themselves, or to transform the carotenoids of their food, inasmuch as they contain carotenoids as yet unknown in the plant kingdom.

Where and in What Forms Carotenoids Occur in Nature. The phytocarotenoids are usually found in the chromatophores, or pigmentary cells, embedded in the plasma. So far at least as lycopene is concerned, there is no evidence that it forms plastids or is produced by their decomposition.⁹²

The color of the chromatophore may be merely that of the carotenoid it contains; or, as so frequently happens, chlorophyll may be present in such overwhelming amount that only its green color is perceived. The green parts of most plants nearly always contain carotene and carotenols, in addition to their chlorophyll. The color of yellow leaves is more often due to carotenoids than to flavones.

In flowers, fruits, and seeds, the amount of carotenoid present varies greatly. Barnes⁹³ found that, on the dry basis, after 145 days' growth, the carotene in carrots amounted to 21.7 mg. per 10 g. Fresh young green lucerne, likewise on the dry basis, contains 40 mg. of carotene per 100 g. of plant.⁹⁴ Of leaf carotenes the major portion is generally the *beta*-isomer.²¹ The *alpha*-form varies from traces up to as high occa-

⁹⁰ Zechmeister and Tuzson, *Z. physiol. Chem.*, **238**, 197 (1936).

⁹¹ Sørensen, *ibid.*, **235**, 8 (1935).

⁹² Smith, *Cornell Univ., Agr. Expt. Sta.*, Mem. **187**, 3 (1936).

⁹³ Barnes, *Cornell Univ., Agr. Expt. Sta.*, Mem. **186**, 36 pp. (1936).

⁹⁴ Myburgh, *Onderstepoort J. Vet. Sci.*, **5**, 475 (1935).

sionally as 35 per cent of the total.⁹⁵ The Perfection pimiento appears to be a rich source of *beta*-carotene, practically free of the *alpha*-isomer, one kilo of the dried shells furnishing 200–665 mg.⁹⁶ In milligrams per kilo, the carotene content of farm feeds has been determined as follows: green grass, 400–650; green alfalfa, 250–400; corn silage, 13–60; etc.⁹⁷ A kilo of fresh bitter nightshade (*Solanum dulcamara*) berries yields about 500 mg. of lycopene; and a kilo of *Euonymus europaeus* seeds, 300–400 mg. of zeaxanthin. In the Chinese lantern plant (alkekengi), 4 kilos of the fresh flower calices contain a total of 10–15 g. of physalien and cryptoxanthin.

Full fertilization, or neon illumination, increases the carotene content of some plants.⁹⁸ Fraps and Kemmerer report that, in the storage of commercial feeds, especially at room or summer temperature, the loss (in both cases high) of vitamin A is much greater than that of carotene.⁹⁹

Carotenoids occur in nature either free or combined. Occasionally they are found in crystalline form, as the carotene in the common carrot root or in the red border of the corolla of *Narcissus poeticus* flowers, but more frequently they are present in colloidal solution in the lipids.

Those found in the free state include the hydrocarbons (lycopene and the carotenes) and the carotenols of green leaves. It is possible that the presence of free carotenols (like xanthophyll) in plants may be due to enzymatic hydrolysis of their esters, but proof of this is still lacking.

Those present in combination are usually in the form of esters (pigment waxes), although a few occur as glycosides (p. 1454) (crocin), or in union with albumins (especially in the zoöcarotenoids). In such esters, the polyene chromophore may be either in the alcohol or in the acid portion of the molecule, and this can be ascertained quickly by hydrolysis, for they are very easily saponified by alkali. In this way, the physalien, $C_{72}H_{116}O_4$, of the alkekengi has been shown to be zeaxanthin dipalmitate, $C_{40}H_{54}(OOC C_{15}H_{31})_2$; and helenien, its isomer, present in a variety of flowers, has been similarly identified as the dipalmitate of xanthophyll. In the yellow pansy flowers there are esters of violaxanthin, in dandelion flowers esters of xanthophyll and of taraxanthin, and in the paprika pods esters of various carotenols with a number of different fatty acids.

How Carotenoids Are Formed in Nature. There are many good reasons for the belief that one of the chief building units utilized by

⁹⁵ Strain, *J. Biol. Chem.*, **111**, 85 (1935).

⁹⁶ Brown, *Science*, **79**, 481 (1934).

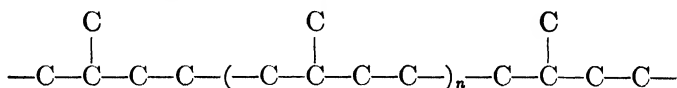
⁹⁷ Shinn, Kane, Wiseman, and Cary, *Proc. Am. Soc. Animal Production*, **27**, 190 (1934).

⁹⁸ Pfützer and Pfaff, *Angew. Chem.*, **48**, 581 (1935).

⁹⁹ Fraps and Kemmerer, *Am. Chem. Soc., Kansas City Meeting* (Apr. 13–17, 1936).

nature in the synthesis of plant products in particular, and to a lesser extent of animal products, is the unsaturated hydrocarbon isoprene, C_5H_8 , or *beta*-methylbutadiene, $CH_2=C(CH_3)CH=CH_2$. The formation of larger molecules from isoprene is assumed to occur in one of the following three ways:

1. By direct linear head-to-tail union,



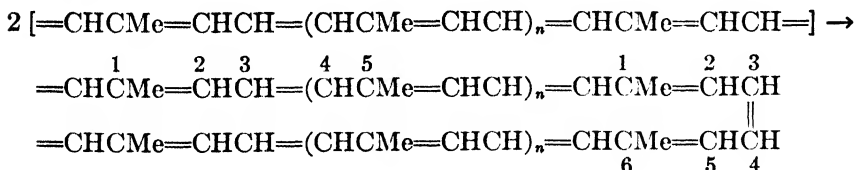
a polymerization which may result in either acyclic or cyclic hydrocarbons, and which is generally regarded as the origin of the terpenes, $(C_5H_8)_n$ (p. 6).

2. By a similar type of polymerization, with concurrent hydrogenation, which would explain the formation of such hydrocarbons as that from which the alcohol phytol, $C_{20}H_{40}O$, is derived.

3. By addition with accompanying dehydrogenation in 1,4-position: $CH_2=CMeCH=CH_2$ less $H_2 \rightarrow =CHCMe=CHCH=$. These C_5H_6 units then combine to longer chains, as in the carotenoids, $=CHCMe=CHCH=(CHCMe=CHCH)_n=CHCMe=CHCH=$, n isoprene units giving $2n + 1$ conjugated double bonds, and it is significant that the number of such bonds in most of the commoner carotenoids is uneven (7, 9, 11, or 13).

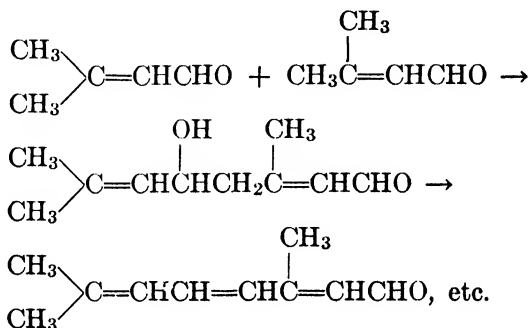
The well-known Diels-Alder diene synthesis (p. 593) has furnished additional support to these theories, by showing under what relatively mild conditions such reactions can be accomplished.

In so far as the carotenoids are concerned, it was early recognized by Karrer and his associates that the direct head-to-tail union of isoprene molecules proceeds only to a very limited extent and that two of the relatively short chains so formed then unite to a longer one, in such fashion that one half mirrors the other and a symmetrical molecule results; or, it has been likened to the construction of a bridge, where the building goes on from both ends at the same time and finally meets in the middle. Where the two come together, the carbons carrying the methyl groups will then bear a 1,6, instead of, as elsewhere, a 1,5 relation to one another:



Further, the ends of the chain, whether cyclic as in *beta*-carotene, or acyclic as in lycopene, are usually less dehydrogenated. In such a process, it is unknown whether the dehydrogenation occurs prior to, during, or after the dimerization; but that it actually takes place is indicated by the oxygen demand of ripening fruits. Very likely it is enzymatic in character.

Another hypothesis, advanced by Karrer⁸ and others, to explain the formation of carotenoids from simpler molecules, assumes *beta*-methylcrotonaldehyde as the building unit.



This has suggested possible methods of synthesizing carotenoids, which have been tried out by several investigators (p. 1207), but without much success as yet.

A problem of obvious interest is the determination of the antecedent C_{20} compounds, from which the C_{40} carotenoids are formed by this dimerization, and Willstätter and Mieg have suggested that phytol, $\text{C}_{20}\text{H}_{40}\text{O}$, might play such a role. Nature's greatest supply of carotenoids is found in the green parts of plants, where they are always associated with chlorophyll. The latter is a phytol ester, about one-third of whose molecule is phytol, and F. G. Fischer's synthesis (p. 1213) of this alcohol has shown it to be composed of four isoprene units in linear head-to-tail union. Further, Karrer and his co-workers¹⁰⁰ (p. 1189) have reduced phytol to dihydrophytol, converted this into the bromide and, by the action of potassium upon the latter, combined two of its dihydrophytyl residues ($\text{C}_{20}\text{H}_{41}$) to a perhydrolycopene, $\text{C}_{40}\text{H}_{82}$, identical with that obtained by the catalytic reduction of the tomato carotenoid, lycopene, $\text{C}_{40}\text{H}_{56}$. Since the carbon skeleton of the two important carotenoid associates of chlorophyll, namely carotene and xanthophyll, $\text{C}_{40}\text{H}_{56}$ and $\text{C}_{40}\text{H}_{56}\text{O}_2$, differs from lycopene only in hav-

¹⁰⁰ Karrer, Helfenstein, and Widmer, *Helv. Chim. Acta*, **11**, 1201 (1928).

ing one or both ends of the chain cyclized, the assumption that phytol is a progenitor of the C_{40} carotenoids seems not unreasonable.

As an additional argument in support of this hypothesis, it might be urged that it has been observed in many cases that the biosynthesis of the carotenoid appears to parallel the gradual disappearance of the chlorophyll; but Kuhn and Brockmann investigated this and found that the quantity of phytol liberated by the chlorophyll decomposed was insufficient to account for all the carotenoid formed. Karrer has suggested that the immediate ancestor may be phytol aldehyde, which is changed into the C_{40} carotenoid by benzoin condensation, or pinacol reduction, with subsequent dehydrogenation.

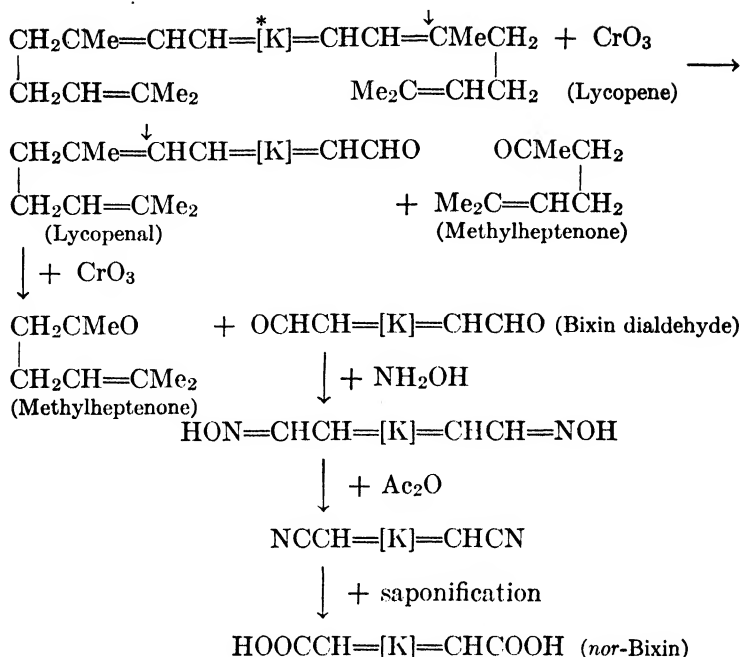
And yet, even granting that phytol is the immediate progenitor of the C_{40} carotenoids, there is good evidence that this phytol does not always come from a breakdown of chlorophyll, for the natural carotenoid synthesis can proceed also in the dark, as illustrated by the formation of carotene in the carrot root, whereas the production of chlorophyll from its colorless antecedents usually requires the action of light. Further, Smith⁹² reports that lycopene was found in mature tomatoes which had been grown in complete darkness and never contained any chlorophyll. Zechmeister, Beres, and Ujhelyi¹⁰¹ gathered flowers from ripening pumpkin plants, while the upper two-thirds of the flowers was yellow and the lower one-third still green. These flowers were cut on the color line and analyzed. In the yellow portion, the carotene was four times and the carotenols three times as abundant as in the green part. The carotenols were zeaxanthin, cryptoxanthin, and xanthophyll. Some colorless substances must be the precursors of at least part of the polyene pigments in the yellow portion of the flowers. A number of the colorless concomitants of phytocarotenoids have been identified by Zechmeister and Tuzson,¹⁰² including various sterols and glycosides.

The carotenoid acids are probably formed by oxidation of the C_{40} carotenoids, for they contain the same central carbon chain. Lycopene, $C_{40}H_{56}$, for example, can be broken down by oxidation with chromic acid, first to lycopenal, $C_{32}H_{42}O$, and then to bixin dialdehyde, $C_{24}H_{28}O_2$, in each case with elimination of a molecule of methylheptanone. This dialdehyde can be converted into the carotenoid acid, bixin, $C_{25}H_{30}O_4$, by the simple reactions shown on p. 1164.

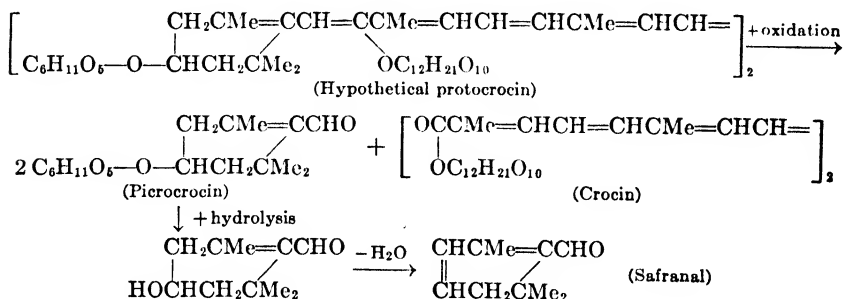
The saffron contains the carotenoid acid, crocetin ("alpha-crocetin"), $C_{20}H_{24}O_4$, its glycoside crocin, and a bitter substance, picrocrocetin, which is likewise a glycoside; but not the monomethyl ("beta-crocetin") or dimethyl ("gamma-crocetin") esters of crocetin, which are formed only

¹⁰¹ Zechmeister, Beres, and Ujhelyi, *Ber.*, **68**, 1321 (1935).

¹⁰² Zechmeister and Tuzson, *Z. physiol. Chem.*, **238**, 204 (1936).



when crocin is hydrolyzed in the presence of methyl alcohol. Kuhn^{103, 104} has suggested that the C₄₀ carotenoid formed in the plant couples first with the sugar, and that this glycoside then oxidizes to one molecule of crocin and two of picrocrocin. The aglucon of the latter, by loss of one molecule of water, passes into the perfume safranal, C₁₀H₁₄O.



Of the 40 carbons in the original carotenoid molecule, 20 would thus be accounted for by one molecule of crocetin and the other 20 by two of

* For the meaning of $=[\text{K}]=$ in these formulas, see p. 1153.

¹⁰³ Kuhn, *Chemistry & Industry*, **52**, 981 (1933) (48 refs.).

¹⁰⁴ Kuhn and Winterstein, *Ber.*, **67**, 344 (1934).

safranal. Experimental tests have shown the presence in the plant tissues of about 1.4 moles of picrocrocin per mole of crocin. The chemical and genetic connection between pigment, bitter substance, and perfume is noteworthy.

Kuhn and Deutsch¹⁰⁵ have suggested that azafrin, a C₂₇ carotenoid acid, may owe its origin to an oxidative cleavage of a C₄₀ carotenoid into the C₂₇ azafrin and ionone (C₁₃H₂₀O).

THE FUNCTION OF CAROTENOIDS IN THE LIVING ORGANISM

Carotenoids in the Plant Kingdom. In the green parts of plants, chlorophyll "a" and "b," carotene, and carotenols, are found in a ratio which remains approximately constant for a given species, but just what is the function of the carotenoid in the metabolism of the plant is still doubtful. Nor can it be confidently asserted that the role of the chlorophyll is yet fully understood.

It has been suggested that the carotenoids are concerned in some way with the breathing or assimilation processes. Since the discovery of the provitamin A effect of carotene in mammals, Virtanen and his collaborators,¹⁰⁶ as well as others, have found that, in plants, the carotene rapidly increases in quantity and concentration up to the beginning of flowering and early fruit setting, and then sinks steadily up to the final ripening of the fruit. In other words, vigor of growth appears to parallel carotene content.¹⁰⁷ There seems to be some evidence also that carotenoids exert an influence upon reproduction in plants.¹⁰⁶ Apparently it may function also as an antioxidant, for the oxidation of a carotene-free sunflower oil, exposed to the air in thin films, is retarded or prevented by the addition of crude carotene.¹⁰⁸

Carotenoids in the Animal Kingdom. Within the spectral range of 280–435 mμ, both carotene and lycopene are said to exert a photodynamic effect upon red blood cells.¹⁰⁹

The fate in the animal organism of ingested phytocarotenoids varies with the particular organism concerned, both as to which pigment is stored and which excreted, and also as to where this storage takes place and what changes the pigment then undergoes.

Mammals, including man, tend to store the carotenes and excrete most of the carotenols.

In horses and cows, the carotene accumulates in the fats and lipids

¹⁰⁵ Kuhn and Deutsch, *Ber.*, **66**, 883 (1933).

¹⁰⁶ See, Murneek, *Science*, **83**, 327 (1936).

¹⁰⁷ Lazar, *Compt. rend. soc. biol.*, **121**, 886 (1936).

¹⁰⁸ Retovsky, *Bull. soc. chim. biol.*, **17**, 1614 (1935).

¹⁰⁹ Kuen and Püringer, *Biochem. Z.*, **286**, 196 (1936).

and gradually imparts its color to them. It is for this reason that such names as "lipochromes" and "chromolipoids" have been used for these pigments. The yellow color of butter is due almost wholly to carotene. It is true that, on the same feed, one breed of cattle may give a yellower butter than another, and the carotene-vitamin A total be the same in both, but since the difference in butter color produced by different feeds is generally much greater than that due to breed alone, the natural yellow color of milk and butter still remains a fairly good index of its vitamin A activity.^{110,111}

In the human body, the carotenes are found chiefly in the liver and fatty tissues, and to a less extent in the kidneys, blood,¹¹² and exudates.

Chromatographic analysis of six human livers by Zechmeister and Tuzson¹¹³ showed, in milligrams per kilo: carotene, 0.20–2.06; lycopene, 0.24–0.54; and xanthophyll, 0.14–0.47.

In human fat, carotenes, lycopene, xanthophyll, and capsanthin have been identified.¹¹⁴ Human colostrum contains very much more carotene and vitamin A than the milk excreted later.^{115,116}

In contradistinction to mammals, birds appear to utilize the carotenols and not the carotenes. The feeding of a carotenoid-free ration to white Leghorn hens results in eggs with nearly colorless yolks. The addition then of xanthophyll or zeaxanthin to the food rapidly restores the yellow color to the yolks. Neither carotene nor lycopene has any such effect. The poultryman thus has it within his power to produce eggs whose yolks will vary in color from a pale yellow to a deep orange, depending upon the demands of his market.

If, after molting, canaries are kept on a carotenoid-free diet, the new feathers will be white. The normal yellow color of the feathers can then be restored gradually, by the addition of xanthophyll or zeaxanthin to the ration, but not by carotene or lycopene.⁶⁹

Sumner and Fox¹¹⁷ have cited evidence which indicates that certain fish change carotene into xanthophyll.

Since the pigment of silkworm cocoons is believed to be carotenoid in character,¹¹⁸ it has been suggested that it may be possible to produce

¹¹⁰ Meigs, *Milk Plant Monthly*, **24**, No. 8, 38 (1935).

¹¹¹ Treichler, Grimes, and Fraps, *Tex. Agr. Expt. Sta., Bull.* **513**, 3 (1935).

¹¹² Clausen, Seidman, and McCord, *Am. Chem. Soc., Kansas City Meeting* (Apr. 13–17, 1936).

¹¹³ Zechmeister and Tuzson, *Z. physiol. Chem.*, **234**, 241 (1935).

¹¹⁴ Zechmeister and Tuzson, *Bull. soc. chim. biol.*, **17**, 1110 (1935).

¹¹⁵ van Eekelen and de Haas, *Geneeskund. Tijdschr. Nederland-Indië*, **74**, 1201 (1934).

¹¹⁶ Neuweiler, *Z. Vitaminforsch.*, **4**, 259 (1935).

¹¹⁷ Sumner and Fox, *Proc. Natl. Acad. Sci. U. S.*, **21**, 330 (1935).

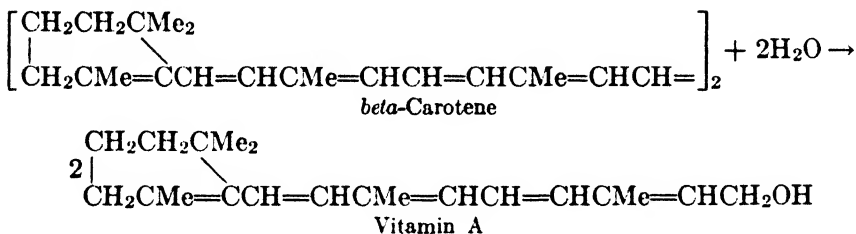
¹¹⁸ Manunta, *Atti soc. nat. mat. Modena*, **66**, 104 (1935); *Boll. soc. ital. biol. sper.*, **12**, 31 (1937).

natural silk of various colors by suitable carotenoid addenda to the usual mulberry-leaf diet of these caterpillars.

Per contra, there are enzymes which possess the power of destroying carotenoid pigments. Thus, an aqueous extract of soybeans is claimed to contain such an enzyme,¹¹⁹ and U. S. patents¹²⁰ have been granted for the bleaching of bread dough by the incorporation therein of a small quantity of such an extract.

Vitamin A. The investigations of recent years have made it clear that carotene is a provitamin A, and that the vitamin A effect of foods runs essentially parallel with their carotene content. Carotene is transformed in the animal body into vitamin A,^{121, 122} which, like carotene itself, tends to accumulate chiefly in the liver. Olcott and McCann have reported that, by the use of liver extracts, they succeeded in converting carotene into vitamin A *in vitro*, and ascribe this to the action of an enzyme which they term "carotenase," but this work still lacks both chemical and biological confirmation. In diabetes, the liver seems to lose some of its power to convert carotene into vitamin A. The absorption of carotene from the blood is thus decreased, with the result that the carotene content of both liver and blood is increased.¹²³

The most obvious explanation of this formation of vitamin A from carotene is that it consists in a simple hydrolysis,¹²⁴ which cuts the carotene molecule in two exactly in the middle.



This constitution for vitamin A was proposed and established by Karrer and his co-workers.^{125, 126} A convincing proof of the structure of the carbon skeleton was provided by the synthesis of perhydrovita-

¹¹⁹ U. S. pat. 2,051,257, Holmes (to Parke, Davis & Co.) (Aug. 18, 1936); [C. A., **30**, 6897 (1936)].

¹²⁰ U. S. pats. 1,957,333 to 1,957,337 inclusive, L. W. Haas and R. M. Bohn (to J. R. Short Milling Co.) (May 1, 1934); [C. A. **28**, 4137 (1934)].

¹²¹ von Euler *et al.*, *Biochem. Z.*, **203**, 370; etc. (1928).

¹²² Ahmad and Drummond, *J. Soc. Chem. Ind.*, 185T (1931).

¹²³ Ralli, Brandaleone, and Mandelbaum, *J. Lab. Clin. Med.*, **20**, 1266 (1935).

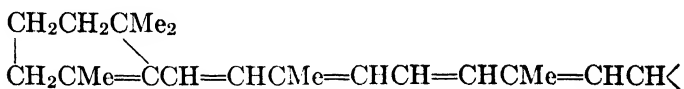
¹²⁴ Schmidt, *Ber.*, **68**, 1658 (1935).

¹²⁵ Karrer *et al.*, *Helv. Chim. Acta*, **14**, 1036 (1931).

¹²⁶ Karrer, Morf, and Schöpp, *ibid.*, **16**, 557 (1933).

min A from *beta*-ionone (p. 1198) and the identity of this synthetic product with a perhydrovitamin A obtained by the catalytic reduction of a carefully purified vitamin A from liver oils.¹²⁶

According to the present state of our knowledge of the connection between structure and vitamin A activity, only those carotenoids which contain the following complex exhibit provitamin A activity, this complex apparently being easily transformed to vitamin A in the living organism.



A change in the location of the double bond in the ionone cycle,¹²⁷ or the insertion of an OH therein, destroys the vitamin A effect. Thus *alpha*-, *beta*-, and *gamma*-carotenes are all active, but the *beta*-isomer, with two such complexes, is approximately twice as potent as the other two. Similarly, the carotenol cryptoxanthin (3-hydroxy-*beta*-carotene) functions as a provitamin A, but rubixanthin (3-hydroxy-*gamma*-carotene) and zeaxanthin (3,3'-dihydroxy-*beta*-carotene), neither of which contains an unhydroxylated *beta*-ionone, are devoid of any such property.

In addition to the three carotenes and cryptoxanthin, the only other naturally occurring carotenoid, and the sole zoöcarotenoid, reported to show vitamin A activity, is the echinenone of the sea urchin.³² Although its constitution is still unknown, it would follow from this property that echinenone contains the vitamin A complex.

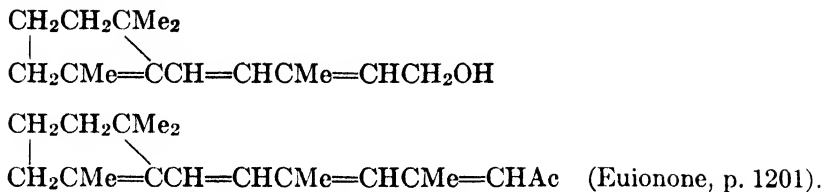
The following synthetic products, however, also show the provitamin A properties:¹²⁸

- beta*-Carotene diiodide, C₄₀H₅₆I₂
- alpha*-Carotene diiodide, C₄₀H₅₆I₂
- Dihydro-*beta*-carotene, C₄₀H₅₈
- Dihydro-*alpha*-carotene, C₄₀H₅₈
- beta*-Carotene oxide, C₄₀H₅₆O
- Oxy-*beta*-carotene, C₄₀H₅₆O₂ (?)
- Semi-*beta*-carotenone, C₄₀H₅₆O₂

Nor do di-, tetra-, or perhydrovitamin A retain the properties of vitamin A, for they have all been synthesized (see p. 1199) and found to be inactive. The following synthetic products are likewise inactive:⁷

¹²⁷ Karrer, v. Euler, and Solmssen, *ibid.*, **17**, 1169 (1934).

¹²⁸ Karrer, *Monatsh.*, **66**, 367-392 (1935).



It is certainly surprising that *beta*-ionone, which is itself devoid of any provitamin A action, should appear to be so essential a part of those polyenes possessing this property; and that a product heretofore of interest chiefly because of its violet perfume should suddenly be discovered to be playing a leading role in the field of carotenoids and vitamins. It is also worth noting how living organisms adapt themselves to their environment and plant and animal cooperate to mutual advantage and for mutual protection. The higher animal organisms, as already mentioned, appear unable to manufacture carotenoids directly, but acquire them from plants and use certain of them for the production of the indispensable vitamin A.

For the use and effects of carotene in cosmetics, see Gattefosse.¹²⁹

Retinene. Human vision seems to depend upon the bleaching of the eye's sensitive "visual purple" by the light,^{130,131} with formation of an orange "visual yellow," whose color is stated to be due to a yellow pigment, related to the carotenoids, termed "retinene" by Wald.^{49,50,51,132,133,134,135,136}

This retinene is liberated from visual purple not only by light, but also by the action of chloroform. It disappears from the retina either by reversion to visual purple or by transformation into vitamin A and other colorless products. The orange color of the visual yellow slowly fades as this change takes place, and the visual purple is regenerated. In the dark, this restoration of visual purple occurs more rapidly and in larger quantity than in the light, thus greatly increasing the sensitivity of the eye, as is evident when one suddenly emerges from a dark room into a bright light.

In this degradation of visual purple to retinene and vitamin A, and its rebuilding from them again, it is not yet clear just how much is due

¹²⁹ Gattefosse, *Parfumerie moderne*, **30**, 473 (1936).

¹³⁰ Hecht, *Science*, **83**, No. 2156, p. 9, Suppl. (1936).

¹³¹ Hecht, Chase, Schlaer, and Haig, *ibid.*, **84**, 331 (1936).

¹³² Wald, *Nature*, **136**, 832 (1935).

¹³³ Wald, *ibid.*, **136**, 913 (1935).

¹³⁴ Verrier and Pannier, *Compt. rend. soc. biol.*, **122**, 600 (1936).

¹³⁵ Wald, *J. Gen. Physiol.*, **20**, 45 (1936).

¹³⁶ Wald, *Science*, **82**, No. 2136, p. 11, Suppl. (1935); *Nature*, **139**, 587 (1937); Jancso and Jancso, *Biochem. Z.*, **287**, 289 (1936).

to the retinene and how much to the vitamin A. Some of the latter is lost in the process, so that the organism requires a constant outside source of supply, for this automatic regulation of the eye's sensitivity does not function properly without it. Miss Tansley¹³⁷ found that, in the dog and rat, vitamin A deficiency caused chemical changes in the eyes which stopped the production of visual purple. One of the first clinical symptoms of vitamin A deficiency in cattle is "night blindness" (nyctalopia),^{56,138} and it is noteworthy that of eighty-five different animal and vegetable Chinese remedies for night-blindness examined by Mar and Read¹³⁹ all were found to be exceptionally rich in vitamin A or provitamin A.

Brunner, Baroni, and Kleinau¹⁴⁰ have isolated *beta*-carotene from the retinas of steers' and pigs' eyes, and they point out that certain similarities in its optical properties to those of visual purple suggest a possible connection between the two. They also found flavin and its photodecomposition products, lumiflavin and lumichrome, to be present.¹⁴¹ A number of cases have been recorded¹⁴² where the oral administration of carotene resulted in improved vision for sufferers from nuclear cataract.

That retinene is the sole, or even the main, source of vitamin A in light-adapted retinas is therefore open to doubt. Until more is known concerning its chemistry, convincing evidence of its distinction from *beta*-carotene, or even that it is a carotenoid at all is lacking.

DETECTION, ISOLATION, PURIFICATION, AND DETERMINATION

Microscopic Examination. The identification of carotenoids by the use of the microscope is rendered difficult by the fact that the crystalline form of these pigments varies greatly depending upon their purity, the nature of the contaminants, the solvents used, and the rapidity of the crystallization. When contaminants have been removed, however, and the pure carotenoids obtained in crystalline form, the microscope is helpful in identifying them and in discovering whether the crystals are all of the same kind or are a mixture of several carotenoids.

Color Reactions. Much of the older literature on the occurrence of carotenes in nature is unreliable, because to the earlier investigators

¹³⁷ Tansley, *Biochem. J.*, **30**, 839 (1936).

¹³⁸ Guilbert and Hart, *J. Nutrition*, **10**, 409 (1935); Jeghers, *Am. Internat. Med.*, **10**, 1304 (1937).

¹³⁹ Mar and Read, *Chinese J. Physiol.*, **10**, 273 (1936).

¹⁴⁰ Brunner, Baroni, and Kleinau, *Z. physiol. Chem.*, **236**, 257 (1935).

¹⁴¹ Brunner, Baroni, and Kleinau, *Ind. Eng. Chem., News Ed.*, **14**, 327 (1936).

¹⁴² Shastid, *Clin. Med. Surg.*, **41**, 492 (1934).

any natural yellow to red pigment which gave a blue color with sulfuric acid was a "carotene." As Molisch has pointed out, this designation should generally be read carotenoid.

The presence of carotenoids can be detected by suitable color reactions, applied either directly to the natural source material or, better, after the carotenoid has been separated and obtained in pure crystalline form. A direct test may sometimes be useful in determining the location of the carotenoid in the tissues.

Reaction with Sulfuric acid. Just a century ago (1835) Marquart observed that when certain yellow flowers were treated with concentrated sulfuric acid, they gave dark blue, bluish violet, or greenish blue colors, and this reaction has been used widely since, as characteristic of carotenoids and as a means of differentiating them from other natural pigments, like the anthocyanins (p. 1114), for example.

Solid substances are generally dissolved in ether or chloroform. The solution is then carefully stratified with concentrated sulfuric acid and the color changes observed in the two layers. Chlorophyll-free plant parts can be tested very simply by rubbing them up in a mortar with fine quartz sand and 5-10 cc. of ether or chloroform, decanting a few cubic centimeters of the solution into a test tube, and stratifying it carefully with 0.5 to 1 cc. of concentrated sulfuric acid. In most cases an 85 per cent acid is sufficiently strong, but in the presence of too much water, the blue color is likely to be immediately replaced by an opaque dirty green.

The chemical significance of this test has found an interesting explanation in the behavior of the synthetic *alpha*, *omega*-diphenylpolyenes with the same reagent, where Kuhn and Winterstein have shown that the color produced depends upon the number of conjugated double bonds in sequence. Thus, in the formula $\text{Ph}-(\text{CH}=\text{CH})_n-\text{Ph}$, when $n = 1$ or 2 , no color is produced by sulfuric acid. Above this, the colors were the following: $n = 3$, yellow-orange; $n = 4$, red; $n = 5$, violet-red; $n = 6$, blue; $n = 7$, blue-green; $n = 8$, blue-green. (See also p. 1178.)

Thus, the presence of at least six conjugated double bonds in direct sequence in the carotenoids is indicated by these results.

In chloroform solution, carotene reacts with formaldehyde in sulfuric acid to form a deep violet zone between the chloroform and acid layers. When the solution is shaken, the color passes into the acid layer, leaving the chloroform colorless.

The Carr-Price Color Reaction (SbCl_3). The deep blue color formed when vitamin A is brought into contact with an anhydrous chloroform solution of antimony trichloride has been recommended by

Carr and Price for the qualitative and quantitative determination of this vitamin. The reaction, however, is not peculiar to vitamin A, but is given likewise by all carotenoids (although in very different degrees). Its chemistry is still obscure. Its velocity varies, but usually the maximum color intensity is reached in 10 to 120 seconds, after which it fades rapidly. The Lovibond tintometer has proved very convenient for making the necessary measurements. The results can be expressed directly in Lovibond "blue values," or calculated to cod-liver oil units (CLO). A compound possesses 1 CLO unit when 20 mg. of it with 1 cc. of Carr-Price antimony trichloride solution gives the same color as 10 Lovibond "blue values."

For certain familiar carotenoids, von Euler and Karrer give the following CLO (± 10 per cent): lycopene, 284; capsanthin, 342; xanthophyll, 432; zeaxanthin, 500; carotene, 508; bixin, 1500.

Rosenthal^{143,144} has found that an addition of guaiacol in this Carr-Price reaction gradually changes the initial blue color to a relatively permanent reddish purple, which is specific for vitamin A.

Colorimetry. In addition to the detection and determination of carotenoids by the Carr-Price color reaction with antimony trichloride, these pigments may also be estimated quantitatively by the color of their solutions in appropriate solvents. Such methods are simple and rapid. They involve merely the comparison of the color of the carotenoid solution with that of the standard solution, in a suitable colorimeter, and are useful also in following the separation and purification of these pigments.¹⁴⁵

The selection of a proper standard solution is of primary importance. Holmes and Bromund¹⁴⁶ have recommended a benzene solution of bixin for the determination of carotene. Instead of pure carotenoids, which are not always available or may not be stable, solutions of inorganic or organic compounds may be employed. Carefully standardized solutions of potassium dichromate, or of organic dyes, have served this purpose.

The British Medical Research Council¹⁴⁷ has recommended as an international unit, for the standardization and determination of vitamin A, 0.0000 mg. of pure *beta*-carotene dissolved in coconut oil, with the addition of hydroquinone as stabilizer.

¹⁴³ Rosenthal, *Klin. Wochschr.*, **14**, 307 (1935).

¹⁴⁴ Rosenthal and Erdelyi, *Biochem. J.*, **29**, 2112 (1935).

¹⁴⁵ Ferguson and Bishop, *Analyst*, **61**, 515 (1935).

¹⁴⁶ Holmes and Bromund, *J. Biol. Chem.*, **112**, 437 (1936).

¹⁴⁷ Hume and Chick, *Med. Research Council (Brit.)*, Special Rept., Ser. No. **202**, 61 pp. (1935).

Kuhn and Brockmann have introduced a microcolorimetric method, using a Hellige microcolorimeter of 1-cc. capacity, by which 0.001 to 0.01 mg. of pigment can be determined. As standard, they use a solution of 14.5 mg. of azobenzene in 100 cc. of 96 per cent ethanol, and compare it with a benzene (b.p. 70–80°) solution of the carotenoid.

In butter fat, the carotene may be determined by means of the photoelectric scopometer.¹⁴⁸

Other Carotenoid Color Reactions. Concentrated hydrochloric acid, with some phenol or thymol, gives a dark blue. The acid alone gives this reaction only with some oxygen-rich carotenoids; with 25 per cent or stronger acid, capsanthin, capsorubin, fucoxanthin, and violaxanthin give positive results.

Fuming nitric acid gives a transient blue color; bromine vapor, a transient dark blue to green.

For color reactions differentiating between carotene, vitamin A, and sterols, see Levine and Bien.¹⁴⁹

Spectroscopic Methods (p. 1180). Spectroscopic observations have been of considerable service in the study of plant pigments, but the value of the older data is often materially reduced through failure to record such important factors as solvent, concentration, thickness of the layer, etc., which influence the position or breadth of the bands. Even to the naked eye, the color of the solution often varies considerably with the solvent. For example, capsanthin gives a brownish yellow solution in ether, but a bluish rose one in carbon disulfide.

The absorption spectra (p. 1142) of the carotenoids are characteristic and easily distinguished from those of most other classes of plant pigments. Thus alcoholic solutions of carotene and xanthophyll, of proper concentration, show two bands in the blue and indigo blue, whose interval is comparable with the breadth of the second band.

It used to be customary to record the boundaries of these bands, but the simpler practice has been adopted recently of giving, in addition to the solvent, only the extinction maxima which, within fairly wide limits, are independent of concentration or thickness of layer. Further, a photometer is not required for these observations since, with sufficiently low concentrations, the bands are symmetrical enough to determine the mean value of their boundaries. If carried out for two different dilutions, placing the crossed hairs in the darkest position, the mean absorption maxima can be obtained (\pm not over 0.5 $m\mu$) with a good grating spectroscope.

¹⁴⁸ Murer, *Proc. 21st Ann. Meeting Western Div. Am. Dairy Sci. Assoc.*, 82–85 (1935).

¹⁴⁹ Levine and Bien, *Proc. Soc. Exptl. Biol. Med.*, **32**, 873 (1935).

The absorption curves for carotene, lycopene, and xanthophyll, obtained by Pummerer, Rebmann, and Reindel, possess the same general shape, thus indicating a close similarity in molecular constitution. For the visible and ultra-violet absorption spectrum of lycopene, see Matlack and Sando.¹⁵⁰ For the absorption spectra of various carotenoids, see Smith and Milner;¹⁵¹ Miller, Mackinney, and Zscheile;¹⁵² Gillam;¹⁵³ and others.¹⁵⁴ As Strain has remarked,⁹⁵ with the exception of adsorption, no properties of carotenoids are more readily determined with small amounts of material than absorption spectra, or wave lengths at maximum absorption.

For very small quantities of pigment, a Hüfner spectrophotometer and Lovibond color filter have been recommended. In mixtures of carotene and vitamin A, the former can be determined conveniently colorimetrically, and the latter spectrophotometrically.^{155, 156}

By means of a new photoelectric spectrophotometric apparatus, Smith¹⁵⁷ has recently measured the absorption spectra of highly purified *alpha*- and *beta*-carotenes, and of lycopene. The absorption coefficients agreed well with the literature for ether-ethanol solutions, but not when the solvent was carbon disulfide.

Chemical Tests. In addition to the color reactions already discussed which are extensively employed as microchemical tests, both qualitatively and quantitatively, carotenoids may be detected either macro- or microchemically by the application of various chemical reagents.¹⁵⁸

The potash method of Molisch rests upon the fact that the carotenoids are resistant to alcoholic alkali, whereas most other natural pigments are decomposed thereby. When an esterified carotenoid is present, as is frequently the case, it too will be hydrolyzed and the product separated will be the constituent carotenoid alcohol or carotenoid acid.

Separation of Mixtures. One of the best methods for the detection, separation, and determination of carotenoids is that depending upon the partition of the pigment between two mutually immiscible solvents. Various methods of recovering carotenes from plant material

¹⁵⁰ Matlack and Sando, *J. Biol. Chem.*, **104**, 413 (1934).

¹⁵¹ Smith and Milner, *ibid.*, **104**, 437 (1934).

¹⁵² Miller, Mackinney, and Zscheile, *Plant Physiol.*, **10**, 375 (1935).

¹⁵³ Gillam, *Biochem. J.*, **29**, 1831 (1935).

¹⁵⁴ Miller, *Plant Physiol.*, **9**, 693 (1934).

¹⁵⁵ Moore, *Chemistry & Industry*, **55**, 834 (1936).

¹⁵⁶ Fr. pat. 760,676, Adam Hilger, Ltd. (Feb. 28, 1934); [*C. A.*, **28**, 4180 (1934)].

¹⁵⁷ Smith, *J. Am. Chem. Soc.*, **58**, 247 (1936).

¹⁵⁸ Murri, *Bull. Applied Botany, Genetics, Plant Breeding* (U.S.S.R.) ser. III, No. 11, **27** (1935).

by the use of suitable mixtures of immiscible solvents have been patented.^{159,160,161}

The lower layer (hypophase) is usually an aqueous methanol, ethanol, or (less frequently) acetone; the upper one (epiphase) is benzine, petroleum ether, petroleum ether + ether, or benzene. Shaken in a separatory funnel with such a mixture of immiscible solvents, most of the carotenoids distribute themselves between the two solvents in definite proportions and, by separation of the layer containing the major portion of the carotenoid sought, the latter may be obtained in practically quantitative yield, if necessary by one or more repetitions of the process. Since the progress of the separation can be followed by the eye, and but little apparatus is required, the method is a simple one. If greater accuracy is desired, it may be used in conjunction with a suitable colorimeter.

In a mixture of dilute methanol and petroleum ether, for example, the C₄₀ carotenoids are distributed so that the carotenes (*alpha*-, *beta*-, and *gamma*-), lycopene, and the pigment waxes pass into the upper layer, whereas those carrying at least two hydroxyl groups (xanthophylls, etc.) will be found in the lower one. By selection of suitable solvents, a complete analytical scheme may be devised for the separation of mixtures of carotenoids.

Capillary Analytical Method. An analytical method based upon the capillarity of carotenoid solutions has often been found useful in determining whether only one or several polyene pigments are present.

When one end of a strip of filter paper is dipped into such an extract, there will appear on the upper non-immersed portion definite zones of characteristic color, breadth, and location, which can be tested separately by chemical reactions, or extracted and examined spectroscopically. Thus, an ordinary leaf extract would show a green chlorophyll zone, with a yellow xanthophyll one above and a yellow carotene one below. Differences in solubility and in adsorbability are largely responsible for these results.

Tswett's Chromatographic Adsorption Method. This method has been excellently described, with 40 literature citations, by Cook.¹⁶² It differs from the method just described in that the adsorbing agent,

¹⁵⁹ U. S. pat. 1,953,607, Holmes and Leicester (to S. M. A. Corp.), April 3, 1934; [C. A. **28**, 3842 (1934)].

¹⁶⁰ U. S. pat. 1,967,121, Holmes and Leicester (to S. M. A. Corp.) (July 17, 1934); [C. A., **28**, 5930 (1934)].

¹⁶¹ U. S. pat. 1,988,031, Barnett (to S. M. A. Corp.) (Jan. 15, 1935), *Off. Gaz.*, **450**, 644 (1935); Claim 1, "A Method of Recovering Carotene from Carrot Oil Which Comprises Adding Several Volumes of Normal Propyl Alcohol to the Carrot Oil and Letting the Mixture Stand to Precipitate Crystalline Carotene;" [C. A. **29**, 1439 (1935)].

¹⁶² Cook, *Chemistry & Industry*, 724 (1936).

instead of being filter paper, is a solid chemical compound (CaCO_3 , Ca(OH)_2 , MgO , Al_2O_3 , sucrose, inulin, basic lead acetate,¹⁶³ etc.). It is regarded by Zechmeister as having been the most valuable contribution, on the physical side, to the chemistry of the carotenoids, since the introduction of spectroscopy.

A vertical glass tube, 10–15 cm. long, 1–2 cm. in diameter, drawn down to a smaller bore at the lower end and connected with a suction flask, is filled with the adsorbent, and a petroleum ether, benzene, or carbon disulfide solution of the material to be tested is introduced at the top and allowed to percolate down. The pigments will thus be fixed in more or less definite zones, in the relative order of their adsorbability. By refilling the tube with pure solvent, a further separation of the zones may be effected. The zones are cut apart mechanically, either by severing the tube or by pushing out the column of adsorbent and dividing it, and are then examined individually, by extraction, colorimetrically or spectroscopically.

The study of adsorptive power in the carotenoid group has shown that this property is greatly influenced by the structure of the molecule. Thus, the adsorption is weakest in the case of the hydrocarbons (carotenes and lycopene) and increases with the number of hydroxyl groups present. Further, for these hydrocarbons themselves, it falls with decrease in the number of double bonds, the carotenes, with 11 or 12 such bonds, being weaker than lycopene, which has 13.

The efficiency of the separation rests to a considerable extent upon the selection of the proper adsorbent and solvent. For the polyene alcohols, calcium carbonate has been found satisfactory; for the hydrocarbons and others, alumina or calcium hydroxide. Strain^{164,165} has recently found that the carotenes can be very satisfactorily separated by this method, using petroleum ether as solvent and magnesium oxide as adsorbent, the *alpha*-carotene moving fastest through the column, then the *beta*-, and then the other carotenes and lycopene.

According to Karrer,¹²⁸ *alpha*-, *beta*-, and *gamma*-carotenes can be separated by fractional crystallization, fractional extraction with methanol, fractional precipitation with iodine, fractional adsorption (on fuller's earth, e.g.), or by the Tswett chromatographic process, the last being the only really satisfactory method. The results depend upon the adsorbent. With calcium hydroxide, the *gamma*-carotene collects in the uppermost zone, then the *beta*-isomer, and below this the *alpha*-carotene.

¹⁶³ Rozenberg, Russ. pat. 40,982 (Jan. 31, 1935); [*C. A.*, **30**, 7285 (1936)].

¹⁶⁴ Strain, *Science*, **79**, 325 (1934).

¹⁶⁵ Strain, *J. Biol. Chem.*, **105**, 523 (1935).

Gillam and El Ridi¹⁶⁶ claim that chromatographic adsorption may cause isomerization of carotenoids, and that by it they have converted *beta*- into *alpha*-carotene, and *vice versa*.

Kuhn and Brockmann's Micro Method. This process of separating and determining carotenoids is a combination of the methods mentioned in the foregoing sections of this chapter. It permits the quantitative estimation of the components of a mixture of carotenoids in small amounts of extract.

Thus, by the application of the partition principle between the two immiscible solvents, benzine and diluted methanol, the hydrocarbons and pigment waxes pass into the benzine layer, the carotenols into the methanol. Saponification of the mixture of hydrocarbons and pigment waxes (carotenol esters) then gives a mixture of the hydrocarbons and carotenols which in turn can be separated by a repetition of the partition process. In the carotenol fraction will be found also any carotenoid acids (bixin, crocetin, azafrin), as well as chlorophyll. The carotenoid acids are recovered by virtue of their solubility in dilute caustic alkali.

From fractions obtained by this partition method, the individual carotenoids are isolated by utilization of the Tswett chromatographic process, and further identified and estimated by spectroscopic, colorimetric, or chemical tests.

For the determination of carotene in fresh plant material, Wiseman and Kane¹⁶⁷ immerse the latter in absolute alcohol cooled to -70° by the addition of solid carbon dioxide and still containing a large excess of this refrigerant. The frozen material is pulverized with a pestle, a large additional quantity of solid carbon dioxide is added, and the mixture is left overnight in a refrigerator at $0-5^{\circ}$. The alcohol is removed by filtration; the solid is suspended in fresh absolute alcohol and ground to a fine powder in a ball mill. The alcohol is again separated by filtration, and the residue washed, first with absolute alcohol and then with ligroin (b.p. $30-60^{\circ}$), after which it is essentially carotene-free. The carotene from the combined filtrates and washings is taken up in ligroin and determined in the usual way.

Guilbert¹⁶⁸ has described a method for the determination of carotene, as a means of estimating the vitamin A value of forage.^{169,170} Peterson, Hughes, and Freeman* have modified this method by using

¹⁶⁶ Gillam and El Ridi, *Nature*, **136**, 914 (1935); *Biochem. J.*, **30**, 1735 (1936).

¹⁶⁷ Wiseman and Kane, *Soc. Biol. Chem., Sci. Proc.* **XXX**, cviii (1936).

¹⁶⁸ Guilbert, *Ind. Eng. Chem., Anal. Ed.*, **6**, 452 (1934).

¹⁶⁹ Guilbert, *J. Nutrition*, **10**, 45 (1935).

¹⁷⁰ Fraps, Treichler, and Kemmerer, *Am. Chem. Soc., Kansas City Meeting* (Apr. 13-17, 1936).

* Kansas City Meeting of the American Chemical Society (Apr. 13-17, 1936).

"Skellysolve B" (b.p. 60–70°), in place of ether, for the extraction of the digested sample, and report consistently higher values.

Elementary Analysis and Molecular Weight Determinations.

Elementary analysis is usually carried out by standard micro methods, keeping in mind the necessity in most cases of having freshly prepared samples, which have not undergone any retrogressive changes due to light, air, or other factors.

Molecular weight is generally determined by the customary cryoscopic or ebullioscopic methods. The camphor micro method of Rast, and the röntgenometric process, are also used, although it has been claimed¹⁷¹ that the Rast procedure gives erroneous results with carotene or xanthophyll.

Specific viscosities of 1.4 per cent solutions of various carotenoids, in carbon tetrachloride or benzene, have been recorded by Staudinger and Steinhöfer.¹⁷²

GENERAL PROPERTIES AND THEIR UTILIZATION IN THE DETERMINATION OF THE CONSTITUTION OF CAROTENOIDS

Crystalline Form. Many of the carotenoids are beautifully crystalline, the form of the crystals varying with the solvent and the conditions of crystallization, as already mentioned (p. 1170). Small quantities of impurities may and do seriously interfere with this crystallization.

X-ray (p. 1758) diffraction powder patterns for a number of carotenoids have been recorded by Mackinney.¹⁷³

Action of Light. In ether solution, at room temperature, carotene is very unstable in the light.¹⁴⁸

Irradiation with ultra-violet light destroys vitamin A within one hour, and carotene within three. Upon this difference is based a procedure for estimating the relative proportion of the two in vegetable and animal foodstuffs.¹⁷⁴

Color. The color of the majority of the carotenoids varies from a bright yellow to a deep red. Some, like rhodoxanthin, are reddish violet to dark blue. It is determined chiefly by the number and distribution of the conjugated double bonds (p. 1211), and by the number and character of the atomic groupings in immediate union therewith.

The fundamentally important investigations of Kuhn and Winterstein^{175,86} on the simple diphenylpolyenes, $\text{Ph}(\text{CH}=\text{CH})_n\text{Ph}$, and of other investigators on related polyenes, have shown clearly the influence of the number of consecutive conjugations upon color.

¹⁷¹ *Carnegie Inst. Year Book*, **26**, 156 (1927).

¹⁷² Staudinger and Steinhöfer, *Ber.*, **68**, 471 (1935).

¹⁷³ Mackinney, *J. Am. Chem. Soc.*, **56**, 488 (1934).

¹⁷⁴ De, *Indian J. Med. Research*, **23**, 505 (1935).

¹⁷⁵ Kuhn and Winterstein, *Helv. Chim. Acta*, **11**, 87, 116, 144 (1928).

TABLE IV

INFLUENCE OF CONSECUTIVE CONJUGATED DOUBLE BONDS UPON VISIBLE COLOR

Ph(CH=CH)Ph	Diphenylethylene (stilbene), colorless.
Ph(") ₂ Ph	" -butadiene, yellowish.
Ph(") ₃ Ph	" -hexatriene, bright greenish yellow.
Ph(") ₄ Ph	" -octatetraene, greenish chrome yellow.
PhCH ₂ (CH=CH) ₄ CH ₂ Ph	Diphenyldecatetraene, colorless.
Ph(CH=CH) ₅ Ph	Diphenyldecapentaene, orange.
Ph(") ₆ Ph	" -dodecahexaene, brownish orange.
Ph(") ₇ Ph	" -tetradecaheptaene, coppery bronze.
Ph(") ₈ Ph	" -hexadecaoctaene, bluish coppery red.
Me(") ₃ COOH	Octatrienic acid, colorless to pale yellow.
Me(") ₄ COOH	Decatetraenic acid, intense yellow.
Me(") ₅ COCOOH	<i>alpha</i> -Ketoheptadienic acid, bright yellow. ¹⁷⁶
Me(") ₆ COCOOH	<i>alpha</i> -Ketononatrienic acid, indian yellow. ¹⁷⁶
Me(") ₇ COCOOH	<i>alpha</i> -Ketoundecatetraenic acid, orange red. ¹⁷⁸
HOOC(CH=CH) ₅ COOH	Hexatriene-1,6-dicarboxylic acid, colorless. ^{177, 178}
HOOCCH ₂ (CH=CH) ₃ CH ₂ COOH	Octatriene-1,8-dicarboxylic acid, colorless. ^{177, 178}
HOOC(CH=CH) ₄ COOH	Octatetraene-1,8-dicarboxylic acid, chrome yellow. ^{177, 178}
HOOC(") ₄ COOH	Decapentaene-1,10- " " , orange " ^{177, 178}
Ph ₂ C=CPh ₂	Tetraphenylethylene, colorless. ¹⁷⁹
Ph ₂ C(=CHCH=)CPh ₂	1,1,4,4-Tetraphenylbutadiene, colorless. ¹⁷⁹
Ph ₂ C(") ₂ CPh ₂	1,1,6,6- " -hexatriene, light yellow. ¹⁷⁹
Ph ₂ C(") ₃ CPh ₂	1,1,8,8- " -octatetraene, yellow. ¹⁷⁹
Ph ₂ C(") ₄ CPh ₂	1,1,10,10- " -decapentaene, orange. ¹⁷⁹
HOOCCH=[K]=CHCOOMe	*Bixin, violet red.
HOOCCH ₂ -[K']-CH ₂ COOMe	Dihydrobixin, yellow.

* For the significance of [K] and [K'], see p. 1153.

Thus, the depth of color increases with the number of these conjugations in immediate and uninterrupted sequence. That the conjugations of the benzene nucleus figure in this is witnessed by the fact that PhCH₂(CH=CH)₄CH₂Ph is colorless, apparently because the CH₂ groups interrupt this sequence between the octatetraene chain and the benzene nuclei.

The carbonyl group, whether present in ketonic or carboxylic function, likewise contributes a double bond which plays a part in determining color.

The dark reddish violet or deep blue color of rhodoxanthin, a carotenoid diketone, has been ascribed to the location of the two carbonyl groups in the terminal cycles in such a position as to increase the number of conjugated double bonds from 12 to 14.

Kuhn and Winterstein have estimated that the effect of a carboxyl

¹⁷⁶ Fischer and Wiedemann, *Ann.*, **513**, 251 (1934).

¹⁷⁷ Kuhn and Grundmann, *Ber.*, **69**, 1757 (1936).

¹⁷⁸ Kuhn and Grundmann, *Ber.*, **69**, 1979 (1936).

¹⁷⁹ Wittig and Klein, *Ber.*, **69**, 2087 (1936).

or phenyl group upon the color, when in direct union with the conjugated straight chain, is equal to that of $1\frac{1}{2}$ ordinary double bonds. Apparently an uninterrupted sequence of at least 5 or 6 double bonds is requisite to endow such compounds with a decided visible color. That a carboxyl is the equivalent of approximately $1\frac{1}{2}$ double bonds is supported by the observation of Kuhn and Hoffer that octatrienic acid, $\text{CH}_3(\text{CH}=\text{CH})_3\text{COOH}$, is colorless to pale yellow, but decatetraenic acid, $\text{CH}_3(\text{CH}=\text{CH})_4\text{COOH}$, is an intense yellow, although the salts of both are colorless. So bixin, with a sequence of 9 conjugated double bonds in the chain, and in direct conjugation at each end with a carboxyl group, has the color equivalent of 12 such bonds. The addition of only 2 atoms of hydrogen to bixin immediately changes its color from violet red to yellow. This hypsochromic effect seems too great to be explained by the simple saturation of a single olefin bond. It has been suggested therefore that one hydrogen atom adds at each end of the hydrocarbon chain, whereby the two terminal groups become $-\text{CH}_2\text{COOH}$ (or $-\text{CH}_2\text{COOMe}$), the CH_2 groups again acting to interrupt, between the hydrocarbon chain and the carboxyl groups, the sequence of conjugations. By the action of methylmagnesium iodide upon this dihydrobixin methyl ester, Karrer and Rübel prepared a tetramethyldihydrobixinol, $\text{C}_{28}\text{H}_{42}\text{O}_2$.

Polarimetric data (p. 200) often are valuable in determining centers of asymmetry in the molecule, for both inactive and optically active carotenoids are known.

Inasmuch as the D line scarcely penetrates a carotenoid solution, Kuhn and his associates have introduced a Siemens-Halske quartz cadmium lamp, with a tube 20 cm. by 10 mm. carrying a current of 4 amperes. Between the lamp and the polarimeter slit are placed a bulb full of water and a Schott and Genossen red filter transmitting monochromatic light of 643.85 μ . As solvent, ethyl acetate, chloroform, or benzene is used, and the measurements are very accurate.

The hot-cathode helium arc of Alex. Rothen has been found especially useful in the measurement of optical rotations.¹⁵¹

Spectroscopic (p. 1765) observations have been of great importance in the study of the carotenoids and in the determination of their constitution.

Ordinarily, the more conjugated double bonds there are united in immediate and uninterrupted sequence, the longer will be the wave length of the maximum light extinction, provided that the molecular structure remains of the same general type. When the absorption bands of two carotenoids practically coincide, it follows that the number and location of the chromophoric unions must likewise be essentially iden-

tical. So, for example, *beta*-carotene, cryptoxanthin, and zeaxanthin are scarcely distinguishable spectroscopically. However, any chemical change in the chromophoric portion of the molecule is promptly reflected in the absorption spectrum. Thus, in the case of ketonic carotenoids, if a decided change in absorption spectra occurs on conversion of the ketone into its oxime, the conclusion is justified that the ketonic carbonyl involved formed part of a conjugated system of double bonds. Where no such change in spectra results from oxime formation, it follows that the carbonyl affected was not part of such a conjugated system. Application of this reasoning to specific carotenoids, both natural and artificial, shows that the reactive carbonyl groups of lycopenal, and of rhodoxanthin, form part of a conjugated system; whereas those of *beta*-carotenone, semi-*beta*-carotenone, and azafrinone do not.

Bisanhydro-*beta*-carotenone (p. 1196) possesses absorption bands of longer wave length than those of rhodoxanthin (p. 1157), although the former has only 11 conjugated double bonds to 12 for the latter. This apparent exception to the general rule is probably to be ascribed to the fact that the one compound contains cyclopentene and the other cyclohexene nuclei. Similar exaltations have been observed in comparing the absorption bands of semi-*beta*-carotenone, *beta*-carotenone, and azafrinone methyl ester, with their corresponding anhydro derivatives.

One peculiarity of those carotenoids which carry a carbonyl or carboxyl group in direct conjugation with a carbon double bond system is the striking distinction between the color of their alcohol and benzene solutions. Usually they give very ill-defined absorption bands in ethyl alcohol, which are of greater wave length than those observed in benzene solution, whereas in all instances where the conjugation is interrupted, the bands in alcohol are clear cut and occupy the same position as in benzene. Thus, rhodoxanthin, capsanthin, and capsorubin are red in alcoholic solution, but orange-yellow in benzene. Kuhn and Brockmann ascribe this to an interaction between the polar carbonyls and the polar alcohol molecules. Dihydrorhodoxanthin does not behave in this way, presumably because, as just explained, its carbonyl groups are separated from the conjugated system by methylene groups. Its maximum absorption, however, now coincides, as does its chromophore, with that of zeaxanthin (see structural formulas, p. 1157), so that its two carbonyl groups have but little to do with the color of the compound.

Fundamentally important contributions on the relation between light absorption and double bonds, as shown by absorption spectra, fluorescence emission spectra, and Raman spectra (p. 1765), have been

¹⁸⁰ Hausser, *Z. tech. Physik*, **15**, 10 (1934).

¹⁸¹ Smakula, *Angew. Chem.*, **47**, 657 (1935).

made by Hausser¹⁸⁰ and by Smakula.¹⁸¹ Their results and graphs show the dependence of such physical properties upon chemical constitution, and have been of the greatest service in deducing the probable structure of the carotenoids.

For quantitative spectral analyses by the spectrophotoelectric method, see the article by Miller.¹⁸²

Action of Heat. Many carotenoids (e.g., lycopene, carotene, xanthophyll) are relatively sensitive to temperatures above 50°, especially when in solution or exposed to the air. Distillations should be carried out therefore in a current of carbon dioxide or of nitrogen, and under such pressures as will avoid high temperatures.

It has been observed that when a carotenoid has been somewhat injured by too high a temperature (or by the air), this injury is not uniformly distributed throughout the mass, but is so localized that the rest of the material can be recovered in pure form by appropriate treatment.

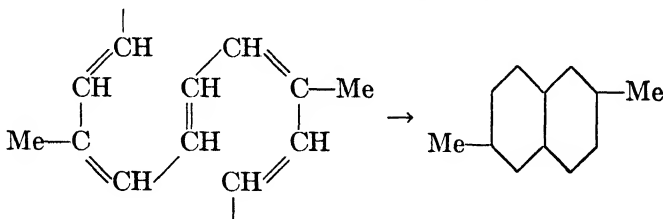
Although the action of heat causes deep-seated decomposition, and the yield of pure products obtained is very small, such products may nevertheless aid in the elucidation of the constitution of the original carotenoid. The methyl groups, for example, are comparatively stable to heat, and their relative positions in the product isolated give a clue to their location in the initial material.

Among the products so isolated have been toluene, *m*-xylene, *m*-toluic acid, 2,6-dimethylnaphthalene, a dicarboxylic acid (C₁₄H₁₈O₄), and tricyclocrocetin (C₂₀H₂₄O₄).

Kuhn and Winterstein have explained the formation of these products as follows:

Toluene arises from cyclization of the =CHCH=CHCMe=CHCH= portion of the chain, and *m*-xylene from a similar cyclization of the =CHCMe=CHCH=CHCMe= part. *m*-Toluic acid, a thermolytic product of carotenoid acids (like bixin), is believed to owe its origin to the final —CH=CHCH=CMech=CHCOOH.

For the 2,6-dimethylnaphthalene, a double cyclization of the middle portion of the carotenoid chain has been held responsible.



¹⁸² Miller, *Plant Physiol.*, **9**, 681 (1934).

Both the tricyclocrocetin and the $C_{14}H_{18}O_4$ acid are products of the action of heat upon crocetin dimethyl ester. The constitution of the former is not known; that of the latter is believed to be $HOCCMe=CHCH=CMcCH=CHCH=CMcCOOMc$.

According to Clausen and McCoord,¹⁸³ in human hyperthermia, there occurs a decrease in plasma carotenoids, but the amount returns to normal (or above) in a few days. Similarly, the vitamin A of the plasma suffers a much greater proportionate destruction by hyperthermia, with subsequent rapid and spectacular recovery to nearly double normal. In both cases, the reserves of the liver, etc., may have been drawn upon to make good the deficiency.

Action of Air. Carotenoids are very susceptible to autoxidation in the air, even at room temperature. Usually it begins gradually, after a certain latent period, and then continues with increasing velocity. It results in loss of color and of crystallizability, gain in weight, and greater solubility. In the solid state or in solution, the individual carotenoids vary widely in their sensitivity. No definite connection has yet been established between constitution and this susceptibility. Traces of catalysts are of potent influence. The purity of the compound has a direct bearing upon its stability. It is possible that the plant protects these air-sensitive constituents in its tissues by the oxygen acceptors commonly present in its cells.

The experiments of Baur¹⁸⁴ indicate that the carotenoids take up oxygen in the light, not merely mole per mole, but by the addition of a large number of moles of oxygen per mole of pigment, resulting in bleaching and complete destruction of the pigment. The reaction takes place in steps. Apparently, first a single mole of oxygen is added, with formation of a "photo-oxide" having a measurable dissociation pressure in the light. Since at least two carotenoids always accompany chlorophyll, the inference is that they are probably concerned with the physiology of assimilation, perhaps as oxygen buffers.

Brief operations, like filtering, drying, etc., can be conducted in the air with relative safety, but those requiring any considerable length of time are better handled in an atmosphere of carbon dioxide or nitrogen. The pure products should be preserved in sealed tubes filled with these same gases. Drying in vacuum desiccators should be over phosphorus pentoxide and not over sulfuric acid.

Permanganate Oxidation (p. 546). By the action of potassium permanganate, under proper conditions, carotenoids can be broken down to

¹⁸³ Clausen and McCoord, *Am. Chem. Soc., Pittsburgh Meeting* (Sept. 7-11, 1936).

¹⁸⁴ Baur, *Helv. Chim. Acta*, **19**, 1210 (1936); **20**, 402 (1937).

various oxidation products which throw light upon the structure of the original molecule.

Thus, *beta*-carotene yields the same oxidation products as *beta*-ionone, namely geronic ($\text{AcCH}_2\text{CH}_2\text{CH}_2\text{CMe}_2\text{COOH}$), *alpha*, *alpha*-dimethylglutaric, *alpha*, *alpha*-dimethylsuccinic, and dimethylmalonic acids. This, in itself, was presumptive evidence of the probable presence of a *beta*-ionone nucleus in the *beta*-carotene molecule, and this was further supported by the strong odor of ionone which filled the whole room where the permanganate oxidation products of carotene were being concentrated.¹⁸⁵

In alkaline solution, permanganate attacks a polyene chain in such a way that the —CMe= groups are oxidized to acetic acid, and the rest to carbon dioxide and water. Determination of the acetic acid then gives a clue to the number of such lateral Me groups present in the original molecule, and each such group may be regarded as representing an isoprene unit.

Chromic Acid Oxidation (p. 546). According to Karrer and his collaborators, chromic acid is more dependable for this purpose than permanganate, which works well only with groups like =CHCMe= and is not satisfactory with such groups as $\text{—CH}_2\text{CMe=}$. Thus the permanganate method applied to crocetin shows only three lateral methyl groups, whereas the chromic acid method shows four.

Kuhn and his associates have made a thorough study of the chromic acid oxidation of carotenoids, and have developed analytical methods, both macro and micro, for the separate determination of the carbon present in lateral methyl groups and in other forms of combination.

In the oxidation of *beta*-carotene by chromic acid, two hydroxyl groups are first added to one of the double bonds in the terminal ionone cycles, with formation of an *alpha*-glycol. Further action of the chromic acid upon this glycol, like that of lead tetraacetate, splits the chain between the two >C(OH)— groups, with production of a diketone. In comparison with potassium permanganate, which likewise adds two hydroxyl groups to a double bond, chromic acid is much more likely to carry the oxidation through to the diketone stage.

The 1,6-diketones which result from this oxidative cleavage of the ionone rings readily undergo cyclodehydration, under the influence of alkali, to cyclopentene derivatives, for this is a general property of 1,6-diketones.

The observation that the preliminary addition of the two hydroxyl groups takes place on the terminal cycles and not on the polyene chain

¹⁸⁵ Karrer and Helfenstein, *ibid.*, **12**, 1142, footnote 5 (1929); Fischer, *Z. physiol. Chem.*, **243**, 103 (1936).

may be explained as due to the fact that such addition to a chain double bond would give a probably much more unstable glycol, and no such hydroxyl derivatives have yet been encountered in natural carotenoids. The hydroxyl groups of the carotenols and related compounds are in the cyclocitral or ionone nuclei, and not on the polyene chain. Kuhn and Brockmann express the opinion that such hydroxylated carotenoids may be intermediate products in the biosynthesis of bixin, crocetin, etc., in plants, whereas, in the animal organism, a phytol type of hydroxylation may be responsible for the generation of two molecules of vitamin A from one of *beta*-carotene. This might occur by the preliminary addition of two hydroxyl groups to the double bond in the middle of the carotene polyene chain, followed by oxidative cleavage of the glycol to two molecules of aldehyde, and finally reduction of the aldehyde to the alcohol (vitamin A) (see, also, p. 1167).

The use of chromic acid in breaking down lycopene first to lycopenal and then to bixin dialdehyde has been cited in discussing the possible origin of the carotenoid acids (p. 1164).

Ozonolysis (p. 546). The presence of the large number of olefin bonds characteristic of the carotenoids renders the latter open to attack by ozone at all these points, and decomposition of the ozonides so formed results in a great variety of products.

The chief service of ozonolysis in this field has been the determination of terminal groups. This concerns particularly those compounds which, like lycopene, carry terminal isopropylidene groups, $=CMe_2$. These groups are split off as acetone molecules, which can then be determined iodometrically.

Applied to lycopene, ozonolysis shows that both ends of the molecule consist of isopropylidene groups, whereas in *gamma*-carotene only one end of the chain has such a terminal group. Thus, lycopene is entirely aliphatic, *gamma*-carotene is aliphatic at only one end, and *alpha*- and *beta*-carotenes are cyclic at both ends.

The ozonolysis of *beta*-carotene yields much the same larger cleavage fragments as *beta*-ionone, while *alpha*-carotene gives both gericonic and isogericonic ($Ac(H_2CMe_2CH_2CH_2COOH)$) acids.

The observation that one molecule of *beta*-carotene yielded two of gericonic acid, whereas *beta*-ionone gives only one, showed that *beta*-carotene must contain two such ionone cycles. Similarly, the first light on the structure of vitamin A was the formation of gericonic acid from it on ozonolysis,¹²⁸ which indicated that it contained an unsubstituted *beta*-ionone cycle.

Unsaturation and Determination of Double Bonds (p. 544). For such highly unsaturated compounds as the carotenoids, it is of first impor-

tance in the study of their constitution to determine the number and location of these double bonds. Their number can be ascertained by the amount of hydrogen, or other addenda, required to saturate them. Their location is shown by the various cleavage products they yield on oxidation, ozonolysis, and similar degradations.

Addition of Hydrogen (p. 545). If carried out under proper conditions catalytic hydrogenation can be depended upon to give a correct measure of the number of olefin bonds present.

As solvents there are employed glacial acetic acid, ethyl acetate, hexane, cyclohexane, decalin, etc.; as catalysts, platinum black, the Adams platinum oxide catalyst, palladium black, palladium barium sulfate, etc. The solvent should be as pure as possible and free from catalyst poisons. Many carotenoids are so difficultly soluble that only very low concentrations can be used, but complete solution usually is unnecessary, since the reduced products are generally more freely soluble, although more time is required for the completion of the saturation in such cases. Occasionally a glacial acetic acid solution can be prepared at 35–40° and if hydrogenated immediately no separation occurs. As a rule, the quantity of catalyst needed for the complete hydrogenation of a carotenoid is relatively large, e.g., 0.5–2.5 g. of platinum per gram of compound.

Of course, there is considerable difference in the behavior of the individual carotenoids on hydrogenation, some losing their color early, others not until near the end of the saturation. Colorimetric hydrogenation curves serve to identify the various polyenes, and these show, for example, the identity of the chromogens of carotene and xanthophyll.

Micro methods, which permit the quantitative determination of double bonds in a few milligrams of substance, have been developed and described by Kuhn and Möller,¹⁸⁶ Slotta and Blanke,¹⁸⁷ Jackson and Jones,¹⁸⁸ and others.

Addition of Halogen (p. 547) may be accomplished by the use of bromine in chloroform solution, but not all the olefin bonds are thus saturated.

Iodine chloride, however, is not often open to this objection, for it usually adds to all the olefin bonds and hence can be used to corroborate the results of hydrogenation.

A U. S. patent¹⁸⁹ has been granted to H. M. Barnett for the recovery of "carotene pigment material" from palm oil, etc., by precipitating

¹⁸⁶ Kuhn and Möller, *Angew. Chem.*, **47**, 145 (1934).

¹⁸⁷ Slotta and Blanke, *J. prakt. Chem.*, [2] **143**, 3 (1935).

¹⁸⁸ Jackson and Jones, *J. Chem. Soc.*, 895 (1936).

¹⁸⁹ U. S. pat. 1,978,981, Barnett (to S.M.A. Corp.) (Oct. 30, 1934) [*C. A.*, **29**, 296 (1935)].

carotene iodide from the gasoline solution by the addition of iodine, and then decomposing this iodide by sodium thiosulfate.¹⁹⁰

Addition of Maleic Anhydride. Carotene and maleic anhydride, in benzene solution, unite to an anhydride, $C_{40}H_{56}(C_4H_2O_3)_5$, m.p. 285–286°, whose absorption spectrum shows no remaining double bonds.^{190a}

Addition of Oxygen. Pummerer and Rebmann have found that

benzoperacid converts a $-\text{CH}=\text{CH}-$ group to $-\text{CH}-\overset{\text{O}}{\text{CH}}-$, but the method labors under the same disability as that of bromination, viz., not all the olefin bonds react. (See also p. 545.)

Determination of Hydroxyl Groups. For this determination, recourse has generally been had to the method of Zerewitinoff (p. 416), which measures the amount of methane evolved when the following reaction is carried out under anhydrous conditions: $\text{ROH} + \text{CH}_3\text{MgI} \rightarrow \text{ROMgI} + \text{CH}_4$. Where more than 3 hydroxyls are present, the results are likely to be erratic and unreliable. Further, enolizable keto groups or other groups or elements which react with the Grignard reagent may interfere.

Roth has worked out a modified micro method, which is carried out in an atmosphere of nitrogen.

Location and Character of the Hydroxyl Groups. As yet primary alcohols are unknown among the carotenoid pigments. Those hydroxylated compounds whose structure is reasonably well known all carry the hydroxyl groups in the cyclic portion of the molecule, and their location therein has been determined by a study of the cleavage products obtained by oxidation, ozonolysis, etc. Thus, ozonolysis of *beta*-carotene yields, among other products, *alpha*, *alpha*-dimethylglutaric acid, whereas xanthophyll fails to give this acid when similarly treated. The hydroxyl in xanthophyll, hence, must be so situated as to prevent the formation of this acid, and a comparison of the graphic formulas of the two (p. 1156) will make this clear.

For the dihydroxylated carotenoids, the method of Criegee¹⁹¹ has proved helpful. This rests upon the observation that 1,2-glycols are smoothly oxidized by lead tetraacetate as follows: $\text{>C(OH)(HO)C<} + \text{Pb(OAc)}_4 \rightarrow \text{>CO} + \text{>CO} + 2\text{AcOH} + \text{Pb(OAc)}_2$, the excess of reagent being back-titrated iodometrically. The olefin bonds of the carotenoid must be hydrogenated before this process can be applied. In this way, Kuhn and Deutsch¹⁹⁵ showed that perhydro-

¹⁹⁰ Ger. pat. 567,783, Kuhn (Mar. 24, 1931) [*C. A.*, **27**, 2698 (1933)].

^{190a} Nakamiya, *Bull. Inst. Phys. Chem. Research* (Tokyo), **15**, 286 (1936).

¹⁹¹ Criegee, *Ber.*, **64**, 200 (1931).

azafrin (like **azafrin** itself) was a 1,2-glycol and that both hydroxyls were tertiary, since the oxidation product possessed no aldehydic properties.

Other methods which have been found useful depend upon the facts that tertiary alcohols are most difficult to esterify and that the oxidation of a perhydro compound to a ketone betokens the presence therein of a secondary alcohol group.

Determination of Methoxyl Groups. This is accomplished by the customary Zeisel process, in either its macro or micro form.

Determination of Carotenoid Esters (Pigment Waxes). These esters may either be investigated directly, or they may be saponified and the resulting alcohol and acid then studied.

The greater solubility of the ester, as compared with its constituent carotenoid, often makes it convenient to hydrogenate it directly and then saponify the perhydro ester. In this way, the complications caused by the unsaturations are avoided, the perhydrocarotenoid obtained by the hydrolysis is readily identified, and the proportion of fatty acid to pigment ascertained. When the fatty acid present is a saturated one, the ester may be used for the determination of its bromine number, its methyl and isopropylidene groups, etc., as well as for the hydrogenation figure.

On account of the sensitivity of the polyenes to mineral acids, the hydrolysis of these esters is carried out always in alkaline media.

Determination of Aldehydic and Ketonic Groups (p. 552). Ketonic groups in direct union with the conjugated system of double bonds can be determined only by the use of free hydroxylamine, at higher temperatures and in the presence of alkali. Analysis of the product indicates the number of ketonic groups present.

But even this process is not always reliable, for occasionally the keto groups fail to form oximes. Thus, the diketones *semi-alpha-* or *-beta-*carotenone give only monoximes, and the tetraketone *beta*-carotenone a dioxime.

In the case of capsanthin, $C_{40}H_{58}O_3$, the fully reduced compound formed a triacetyl, whereas the original pigment yielded only a diacetyl derivative. From these observations, it was concluded that this carotenoid was a dihydroxy ketone, although it was not found possible to prepare an oxime from it.

Judged by the behavior of lycopenal, polyene aldehydes readily form oximes; but no aldehydic carotenoids have yet been discovered in nature.

In the matter of oxime formation, Kuhn and Brockmann⁵ have arranged carotenoid aldehydes and ketones, in the order of their reactivity with hydroxylamine, as follows:

1. React readily: Aldehydes of $-(C=C)_n-CHO$ structure; and

ketonic groups of true aliphatic type, like the $-\text{CMe}_2(\text{CH}_2)_3\text{COMe}$, resulting from the opening of a cyclohexene nucleus.

2. React with difficulty: The conjugated keto groups of rhodoxanthin, anhydro-semi-beta-carotenone, bisanhydro-beta-carotenone, and anhydroazafrinone.

3. Do not react: Conjugated keto groups united with a quaternary carbon, e.g., $-\text{CMe}_2\text{COCH}=\text{CH}-$.

Determination of carboxyl groups is carried out by the usual titration methods, or by esterification with diazomethane.

Stereochemistry (p. 367). The many double bonds of the polyene pigments suggest the possibility of various *cis-trans* isomers, although, in the light of our modern valence theory, the tendency to such isomerism should decrease with increase in the number of conjugations.

The fact that the synthetic diphenylpolyenes of Kuhn and Winterstein are known in but one form has been explained on the basis that their method of preparation results necessarily in the production of the stablest (i.e., the *trans*-) configuration, and this is supported by röntgenometric determinations. Most of the natural carotenoids, with the exception of *cis*-crocetin and bixin (*cis*-), likewise appear to possess the *trans*-configuration.

It has been suggested that, in the early stages of the plant, the *cis* (labile) forms occur, and that, as the buds open and the rays of the sun enter, these *cis* forms rearrange to the stabler *trans* isomers.

Of the various carotenols, flavoxanthin, taraxanthin, violaxanthin, and xanthophyll are dextrorotatory; cryptoxanthin, fucoxanthin, rubixanthin, and zeaxanthin are optically inactive.

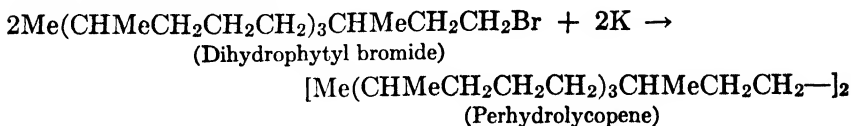
THE CONTRIBUTIONS OF SYNTHETIC CHEMISTRY

None of the natural carotenoids has been synthesized as yet, and this is not surprising in view of the long branched carbon chain and the extensive system of conjugated unsaturations.

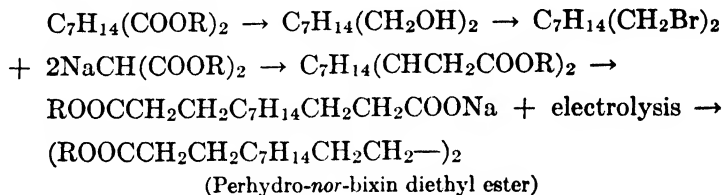
The complete carbon skeleton deduced for certain of the carotenoids, however, has been corroborated by the synthesis of *perhydrolycopene*, identical with the catalytic reduction product of the lycopene separated from the tomato; of *perhydro-nor-bixin diethyl ester*, identical with the reduction product of *nor-bixin* diethyl ester obtained by esterification of natural bixin; and of *perhydrocrocetin*, identical with the reduction product of the polyene pigment of the saffron. These syntheses were accomplished as follows.

Perhydrolycopene, $\text{C}_{40}\text{H}_{82}$, was obtained by Karrer, Helfenstein, and

Widmer,¹⁰⁰ by the action of metallic potassium (or magnesium) upon dihydrophytyl bromide:



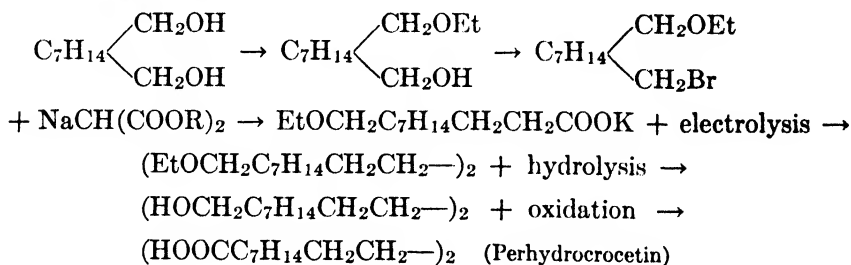
Perhydro-nor-bixin diethyl ester, $\text{C}_{28}\text{H}_{54}\text{O}_4$. The initial material was *alpha, alpha'*-dimethylpimelic ester, $\text{ROOC} \downarrow \text{CHMeCH}_2\text{CH}_2\text{CH}_2\text{CHMe} \downarrow \text{COOR}$. For convenience, that portion of this formula between the two arrows is represented as C_7H_{14} in what follows:



This synthesis was accomplished by Karrer, Benz, Morf, Raudnitz, Stoll, and Takahashi.²⁷

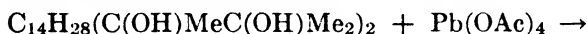
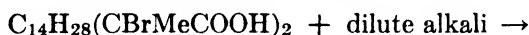
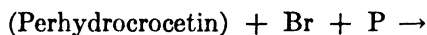
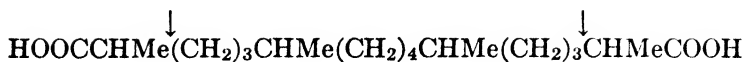
The constitution assigned to bixin is further supported by the fact that bixin methyl ester on ozonolysis yields $\text{HOCCH}=\text{CMeCH}=\text{CHCOOMe}$ (*beta*-methylmuconic ester) and $\text{MeCOCH}=\text{CHCOOMe}$ (acetylacrylic ester).

Perhydrocrocetin, $\text{C}_{20}\text{H}_{38}\text{O}_4$. Starting with 2,6-dimethylheptandiol-1,7, $\text{HOCH}_2 \downarrow \text{CHMeCH}_2\text{CH}_2\text{CH}_2\text{CHMe} \downarrow \text{CH}_2\text{OH}$, obtained by reduction of the *alpha, alpha'*-dimethylpimelic acid noted above, and again representing by C_7H_{14} that portion of the formula between the two arrows, the successive steps taken by Karrer, Benz, and Stoll¹⁹² in carrying out this synthesis were the following:

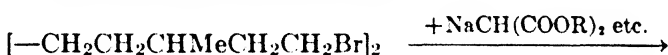
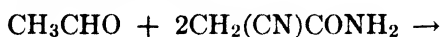


Perhydrocrocetin²⁷ has been degraded to a diketone by the following steps, using $\text{C}_{14}\text{H}_{28}$ to represent that portion of the perhydrocrocetin formula between the two vertical arrows:

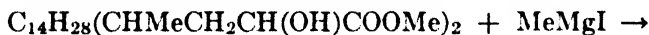
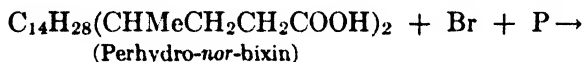
¹⁹² Karrer, Benz, and Stoll, *Helv. Chim. Acta*, **16**, 297 (1933).



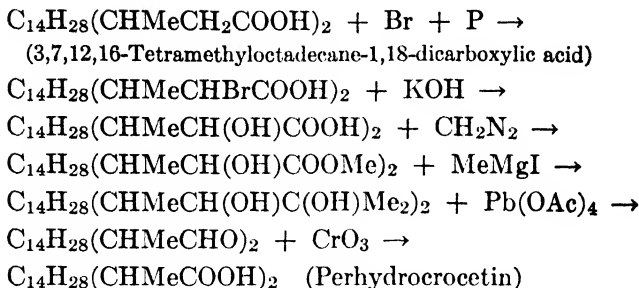
$\text{C}_{14}\text{H}_{28}\text{Ac}_2$ (6,11-Dimethylhexadecane-2,15-dione) and this diketone has been synthesized thus:



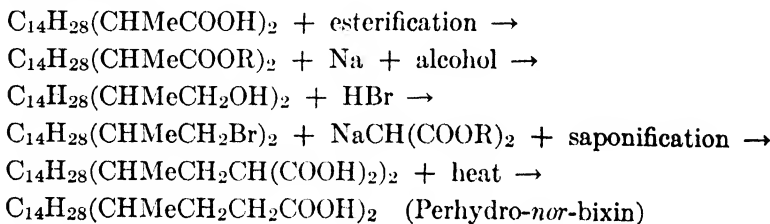
Natural perhydro-*nor*-bixin has been broken down to a dialdehyde,²⁷ and the acid obtained from the latter by oxidation (3,7,12,16-tetramethyloctadecane-1,18-dicarboxylic acid) has in turn been built up to perhydrocrocin,¹⁹³ the successive steps being these:



¹⁹³ Raudnits and Peschel, *Ber.*, **66**, 901 (1933).



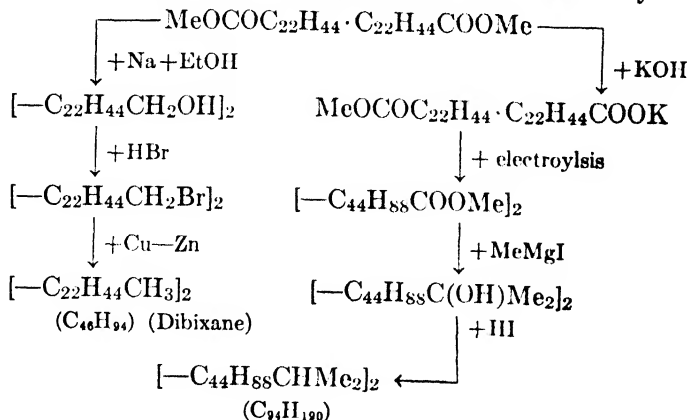
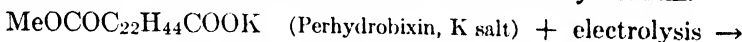
Further, natural perhydrocrocetin has been built up to perhydro-*nor*-bixin thus:¹⁹⁴



The paraffin hydrocarbons corresponding to crocetin and bixin have been named by Karrer "crocetane" (2,6,11,15-tetramethylhexadecane) and "bixane" (4,8,13,17-tetramethyleicosane), while "dibixane" is the 4,8,13,17,22,26,31,35-octamethyloctatriacontane.

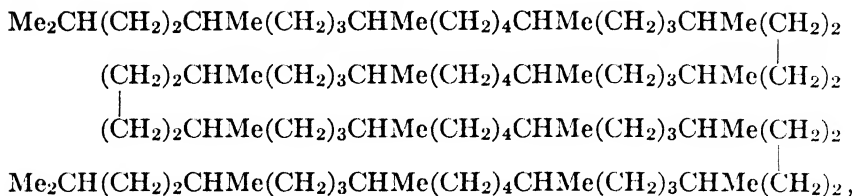
From the potassium salt of perhydrobixin, Karrer, Stoll, and Stevens¹⁹⁵ carried out the following syntheses:

Syntheses of Higher Hydrocarbons from Perhydrobixin.



¹⁹⁴ Karrer and Benz, *Helv. Chim. Acta*, **16**, 337 (1933).

¹⁹⁵ Karrer, Stoll, and Stevens, *ibid.*, **14**, 1194 (1931).



which is chemically 2,5,9,13,17,24,28,32,36,41,45,49,53,60,64,68,72,75-octadecamethylhexaheptacontane, and is the highest paraffin hydrocarbon of known structure yet synthesized.

The Constitution of *beta*-Carotene and of Azafrin. The structural formula for *beta*-carotene, proposed by Karrer and Morf,¹⁹⁶ which makes it a 7,7'-bis[1,1,5-trimethylcyclohexene-5-yl]-9,9',13,13'-tetramethyloctadecanonane, was deduced from the following experimental data:

1. The molecular formula was established as $\text{C}_{40}\text{H}_{56}$ by Willstätter and Mig.¹⁹⁷

2. Zechmeister, von Chohnoky, and Vrabely,¹⁹⁸ by catalytic hydrogenation, demonstrated the presence therein of eleven double bonds and two cycles.

3. By chromic acid oxidation, Kuhn and his associates¹⁹⁹ determined that there were four methyl side chains in the molecule.

4. Karrer and his co-workers,²⁰⁰ by permanganate oxidation, succeeded in breaking it down to geronic ($\text{AcCH}_2\text{CH}_2\text{CH}_2\text{CMe}_2\text{COOH}$), *alpha, alpha*-dimethylglutaric ($\text{HOOCCH}_2\text{CH}_2\text{CMe}_2\text{COOH}$), *alpha, alpha*-dimethylsuccinic ($\text{HOOCCH}_2\text{CMe}_2\text{COOH}$), and dimethylmalonic acids ($\text{HOOCMe}_2\text{COOH}$), and obtained at the same time evidence of the formation of *beta*-ionone (p. 1184). Further, the fact that one mole of *beta*-carotene yielded two of geronic acid, whereas one of *alpha*-carotene gave one of geronic and one of isogeronic acid ($\text{AcCH}_2\text{CMe}_2\text{CH}_2\text{CH}_2\text{COOH}$), led to the natural conclusion that *beta*-carotene contained two *beta*-ionone cycles, and *alpha*-carotene one *alpha*- and one *beta*-ionone cycle.

5. It behaves as a provitamin A, and the synthesis of perhydrovitamin A by Karrer and his collaborators has been accomplished.¹²⁶

6. The physical properties of the compound, especially its optical behavior agree.

7. The series of degradation and synthetic reactions (pp. 1194-95), car-

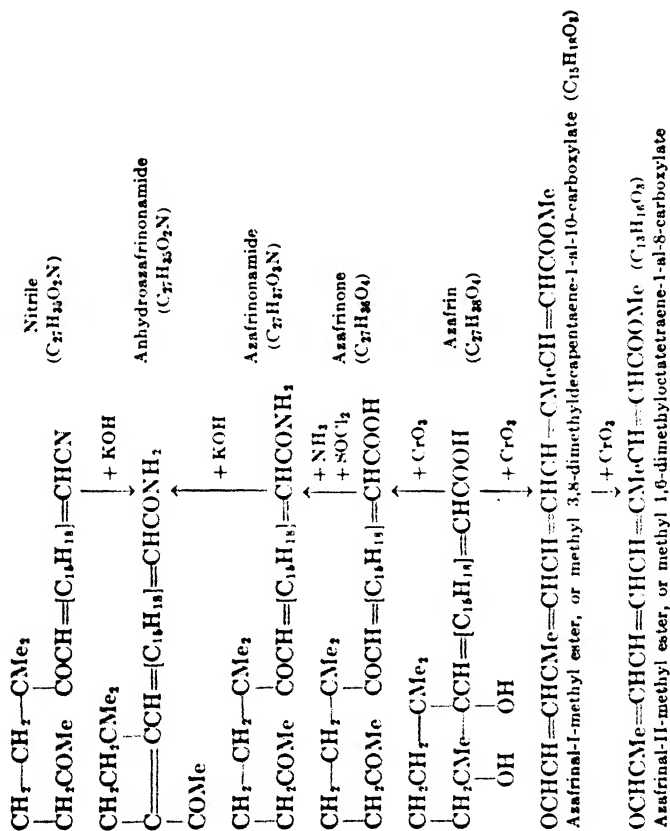
¹⁹⁶ Karrer *et al.*, *ibid.*, **14**, 1431 (1931).

¹⁹⁷ Willstätter and Mig, *Ann.*, **355**, 1 (1907).

¹⁹⁸ Zechmeister, v. Chohnoky, and Vrabely, *Ber.*, **66**, 123 (1933).

¹⁹⁹ Kuhn and Brockmann, *Ber.*, **67**, 885 (1934).

²⁰⁰ Karrer and Helfenstein, *Helv. Chim. Acta*, **12**, 1142 (1929).



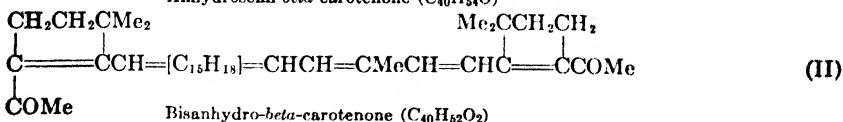
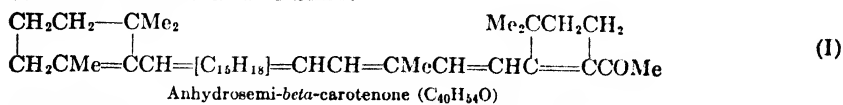
ried out by Kuhn and Brockmann,^{5,199,201} throw light not only upon the structure of *beta*-carotene, but also upon that of azafrin. In these reactions, the $\equiv[C_{15}H_{18}]\equiv$ represents that portion of the *beta*-carotene formula between the two vertical arrows. (See equations on pp.1194-95)

The non-production of isomers in this oxidative breakdown of the *beta*-carotene molecule is in itself a corroboration of the symmetrical constitution of the polyene chain. This fact, together with the identity of the products formed by oxidation, as well as of those resulting from the action of heat, shows that, except for the four lateral methyl groups, the polyene chain carries no other branches. The location of the four lateral methyls also follows from these experimental observations.

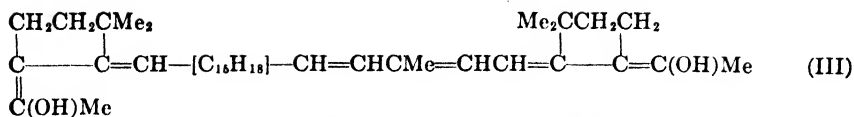
The aldehydes and acids which result from the oxidation of azafrin prove the structure of the polyene chain in this carotenoid, and the production of one and the same anhydroazafrinonamide from both azafrin and *beta*-carotene is evidence that the polyene chain in the two must be the same.

It was found impossible to oxidize *beta*-carotenonaldehyde direct to the corresponding acid (azafrinone). Similarly, the aldehyde ester, $C_{15}H_{18}O_3$, obtained by the action of chromic acid upon azafrin, could not be oxidized direct to the corresponding acid, but was converted into it by way of the oxime, nitrile, and saponification. This dibasic acid, $C_{14}H_{16}O_4$, is a lower homolog of crocetin, crystallizes in small yellow needles (m.p. 267-268°), and is the first polyene dicarboxylic acid with 5 conjugated double bonds.

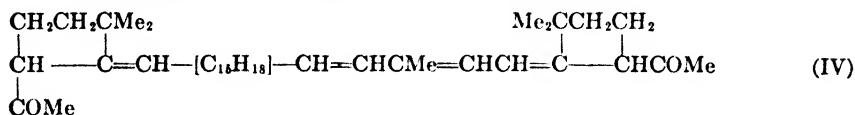
Just as azafrinonamide, under the influence of alkali, passes into anhydroazafrinonamide, so semi-*beta*-carotenone and *beta*-carotenone yield the corresponding anhydro derivatives (I and II). Reduction of (II) gives a dihydro derivative, in which it is assumed that the addition of hydrogen occurs at the ends of the conjugated system, with formation first of a dienol (III), which then rearranges to the stabler diketone (IV). By atmospheric oxygen, this dihydro derivative can be reconverted into the initial compound (II). A similar reversible reduction and oxidation has been observed in *beta*-carotenone, rhodoxanthin, and the methyl esters of crocetin and of bixin.



²⁰¹ Kuhn and Brockman, *Ber.*, **67**, 1408 (1934).



Bisanhydro-dihydro-beta-carotenone (dienol) ($C_{40}H_{54}O_2$)

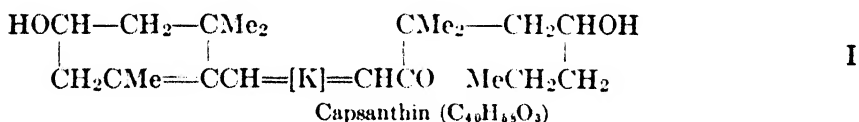


Bisanhydro-dihydro-beta-carotenone (diketo) ($C_{40}H_{54}O_2$)

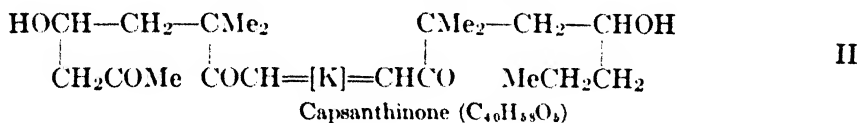
Karrer and Solmssen have obtained *beta*-carotenal as a degradation product of *beta*-carotene.^{201a}

The Constitution of Capsanthin (p. 1143). The partial degradation of the capsanthin (I) molecule, by a similar careful oxidation with chromic acid, has been reported recently by Zechmeister and v. Cholnoky.¹⁹ Instead of the free carotenoid, its more suitable diacetate served as initial material.

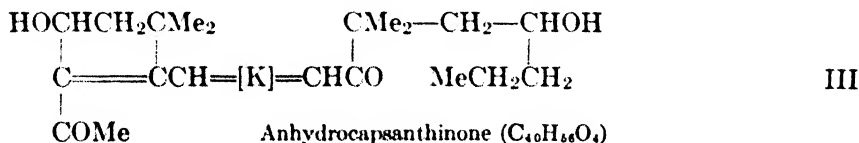
The first oxidation product isolated was the diacetate of capsanthinone (II) which, under the influence of alkali, closed up to the anhydrocapsanthinone (III). More vigorous oxidation gave a mixture of capsanthylal (IV), capsyl aldehyde (V), and hydroxy-*beta*-carotenone aldehyde (VI), which were separated chromatographically. The cyclic end of capsanthin is believed to be now well established, but the structure of the acyclic end is still in doubt.



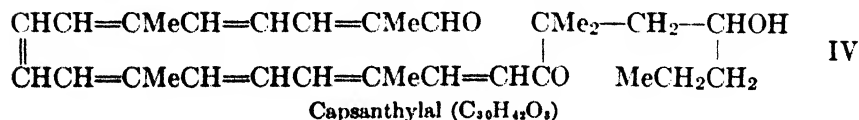
Capsanthin (C₄₀H₅₆O₃)



Capsanthinone (C₄₀H₅₈O₆)



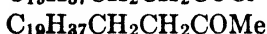
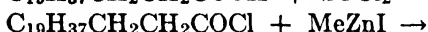
Anhydrocapsanthinone (C₄₀H₅₆O₄)



Capsanthylal ($C_{30}H_{42}O_8$)



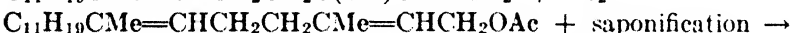
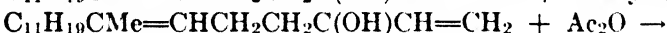
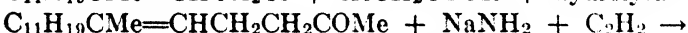
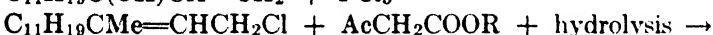
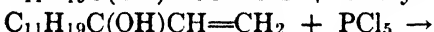
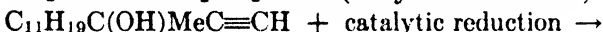
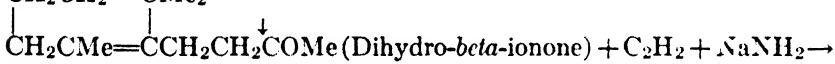
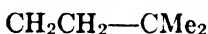
(Perhydrovitamin A)



The melting point of the above acid, and of the semicarbazone of the ketone obtained therefrom, were the same in the two series, and the mixed melting points were also the same.

Octahydrovitamin A. Kawakami²⁰³ has hydrogenated a crude vitamin A from cod-liver oil and distilled the product at 5 mm. pressure. The main fraction, b.p.₅ 180–190°, purified through its acid phthalate, gave the octahydro derivative, C₂₀H₃₈O, b.p. 190° at 7 mm., d_4^{20} 0.90565, and n_D^{20} 1.487.

Tetrahydrovitamin A [1-(*beta*-Cyclogeranyl)-geraniol]. Ruzicka and Fischer²⁰⁴ have prepared a tetrahydrovitamin A by the following series of reactions, using both *alpha*- and *beta*-ionones as initial materials.



Purified through their phthalic esters, they formed viscous oils, b.p. 136–138° at about 0.1 mm. pressure. Ruzicka has patented them.^{205, 206, 207, 208} Like perhydrovitamin A, they are without any of the growth-stimulating properties of vitamin A itself.

Dihydrovitamin A. Gould²⁰⁹ has recently completed the synthesis

²⁰³ Kawakami, *Sci. Papers Inst. Phys. Chem. Research* (Tokyo), **26**, 77 (1935).

²⁰⁴ Ruzicka and Fischer, *Helv. Chim. Acta*, **17**, 633 (1934).

²⁰⁵ U. S. pat. 1,999,110, Ruzicka (to Soc. pour l'ind. chim. à Bâle) (Apr. 23, 1935); [*C. A.*, **29**, 4021 (1935)]; Ger. pat. 601,070, Soc. pour l'ind. chim. à Bâle (Aug. 16, 1934); [*C. A.*, **28**, 7261 (1934)].

²⁰⁶ Ger. pat. 601,070, Soc. pour l'ind. chim. à Bâle (Aug. 16, 1934); [*C. A.*, **28**, 7261 (1934)].

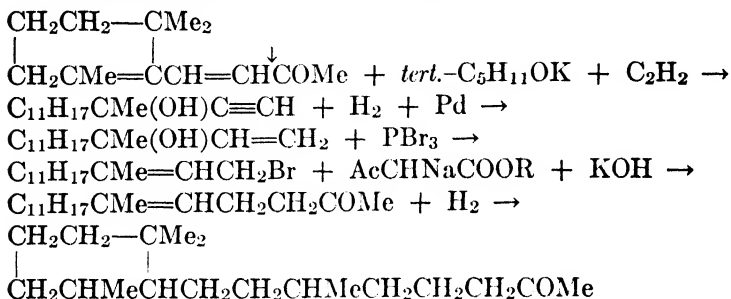
²⁰⁷ Swiss pat. 168,135, Soc. pour l'ind. chim. à Bâle (June 1, 1934); [*C. A.*, **29**, 552 (1935)].

²⁰⁸ Swiss pat. 174,869, Soc. pour l'ind. chim. à Bâle (April 16, 1935); [*C. A.*, **30**, 249 (1936)].

²⁰⁹ Gould, *J. Biol. Chem.*, **114**, xli (1936).

of a dihydrovitamin A, which he began with Thompson,²¹⁰ using *beta*-ionone as initial material. A noteworthy discovery in the prosecution of the work was that, in the Nef reaction, potassium *tert*.-butylate or *tert*.-amylate could be used advantageously in place of sodium or sodium amide.

The synthesis involved these steps:



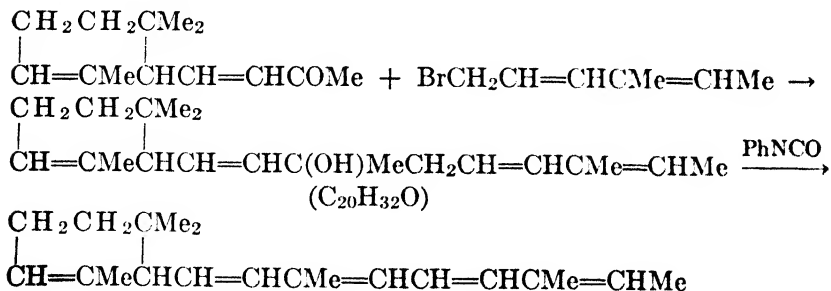
By a similar series of reactions, this last compound was synthesized also from tetrahydroionone.

The synthesis was then continued by applying the Nef reaction ($\text{C}_2\text{H}_2 + \text{tert.}-\text{C}_5\text{H}_{11}\text{OK}$) to the next to the last compound above, and this yielded



$\text{C}_{11}\text{H}_{17}\text{CMe}=\text{CH}(\text{CH}_2)_2\text{CMe}=\text{CHCH}_2\text{OH}$, which was purified through the phthalate and also turned out to be devoid of vitamin A activity.

Milas and McAlevy²¹¹ have condensed the magnesium derivative of 1-bromo-4-methyl-2,4-hexadiene with *alpha*-ionone, and dehydrated the product by phenyl isocyanate or potassium hydrogen sulfate. There resulted a deep red hydrocarbon, whose chemical properties resembled those of carotene and of vitamin A, and for which they suggested the formula shown below.



²¹⁰ Gould and Thompson, *J. Am. Chem. Soc.*, **57**, 340 (1935).

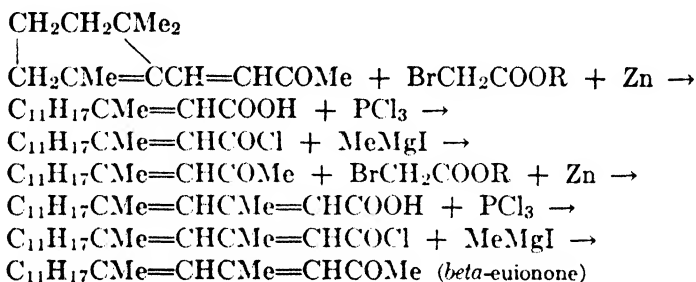
²¹¹ Milas and McAlevy, *ibid.*, **57**, 580 (1935).

If the pure hydrocarbon is highly colored, it can scarcely possess this formula, since four consecutive conjugations are not sufficient to endow it with any such property (p. 1171).

The physiological effects of the tertiary alcohol have not been reported.

Kuhn and Morris have reported recently the synthesis from *beta*-ionylideneacetaldehyde of a vitamin A of approximately 7.5% purity.^{211a}

***beta*-Euionone.** Another interesting synthesis in this field was that of *beta*-euionone, by Karrer and Morf,²¹² using *beta*-ionone as initial material:



This final product was without any vitamin A action when administered to animals, but possessed a finer and more powerful violet perfume than the ionones.

Crystalline Derivatives of Vitamin A. Vitamin A has been obtained usually as a pale yellowish viscous oil, whose ultra-violet absorption spectrum shows a maximum at 328 m μ . Spectrophotometric methods of estimating vitamin A by means of the extinction coefficient of 328 m μ , alone or in the presence of carotene, have been developed by Morton and Heilbron,^{213,214,215} Drummond and Morton,²¹⁶ and by Coward *et al.*²¹⁷ A new photoelectric method for measuring vitamin A has been described by McFarlan, Reddie, and Merrill.^{217a} By an improved technique, Holmes and his co-workers^{218,218a} have prepared highly

^{211a} Kuhn and Morris, *Ber.*, **70**, 853 (1937).

²¹² Karrer and Morf, *Helv. Chim. Acta*, **17**, 3 (1934).

²¹³ Morton and Heilbron, *Nature*, **123**, 10 (1928).

²¹⁴ Morton and Heilbron, *Biochem. J.*, **23**, 988 (1928).

²¹⁵ Morton and Heilbron, *ibid.*, **24**, 870 (1930).

²¹⁶ Drummond and Morton, *ibid.*, **23**, 785 (1929).

²¹⁷ Coward *et al.*, *ibid.*, **25**, 1102 (1931).

^{217a} McFarlan, Reddie, and Merrill, *Ind. Eng. Chem. (Anal. ed.)*, **9**, 324 (1937).

²¹⁸ Holmes *et al.*, *J. Am. Chem. Soc.*, **57**, 1990 (1935).

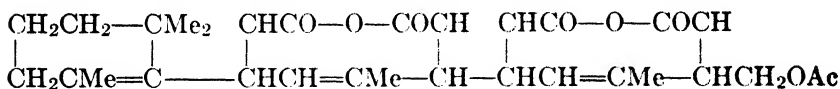
^{218a} Holmes and Corbet, *Science*, **85**, 103 (1937).

potent vitamin A concentrates, culminating in the securing of a crystalline concentrate from the liver oil of *Stereolepis ishinagi*, of approximately 100,000 blue values and $E_{1\text{cm}}^{1\%} = 2000$ (by Hilger Vitameter-A). The pale yellow crystals, formed at low temperature, melted at $5.5\text{--}6^\circ$ to a viscous yellow liquid.

Terada,²¹⁹ by heating a mixture of crude vitamin A and succinic anhydride, obtained an *acid succinate*, as a reddish yellow oil, which, after saponification with sodium hydroxide, yielded vitamin A as a yellowish oil.

There has been great need therefore of stable crystalline derivatives, which could be identified by their analysis and the determination of their melting points and other physical constants. The following contributions in this direction have recently been published by Japanese investigators.

Kawakami,²⁰³ starting with a vitamin A concentrate from the liver oil of *Theragra chalcogramma*, condensed its acetate, in benzene solution and in an atmosphere of carbon dioxide, with two molecules of maleic anhydride, thereby obtaining acetylsukesoic acid anhydride (I), $\text{C}_{30}\text{H}_{36}\text{O}_8$, m.p. $261\text{--}262^\circ$, to which he assigned this formula:



The corresponding benzoyl derivative, $\text{C}_{35}\text{H}_{38}\text{O}_8$, melted at $263\text{--}264^\circ$.

Using an acetylated vitamin A, prepared from the liver oil of *Stereolepis ishinagi*, he obtained by the same process an *acetylishnagiic anhydride* (II), m.p. $221\text{--}222^\circ$, isomeric with (I) and believed to have a similar structure.

Hamano,²²⁰ using a chromatographically purified vitamin A, of 8300 CLO, repeated Kawakami's work and confirmed his results. He also discovered that, when he treated the fresh liver oil of *Stereolepis ishinagi* directly with maleic anhydride, in benzene solution at $90\text{--}100^\circ$ in an atmosphere of carbon dioxide until the antimony trichloride reaction was negative, there resulted a crystalline product, $\text{C}_{44}\text{H}_{64}\text{O}_8$, m.p. 220° , which was the *palmitylvitamin A-maleic anhydride adduct* (III) since, when saponified by alkali, it yielded palmitic acid and *vitamin A-dimaleic acid lactone* (IV), $\text{C}_{28}\text{H}_{36}\text{O}_8$, m.p. 184° . The constitution of (III) was corroborated by its synthesis from a purified vitamin A concentrate, heated first with palmityl chloride and then with maleic anhy-

²¹⁹ Jap. pat. 101,491, Terada (June 13, 1933) [*C. A.* **28**, 5182 (1934)].

²²⁰ Hamano, *Sci. Papers Inst. Phys. Chem. Research* (Tokyo), **26**, 82, 87 (1935).

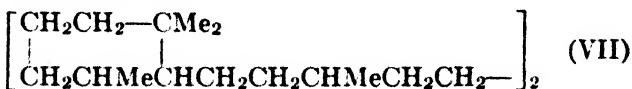
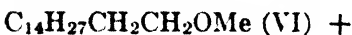
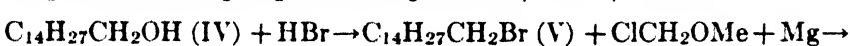
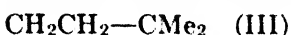
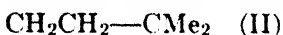
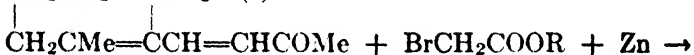
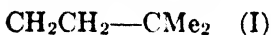
dride. It follows that the vitamin A in this liver oil, and quite probably in other fish-liver oils as well, is present as the palmityl ester.

As Hamano points out,²²¹ none of the above crystalline derivatives are physiologically active, nor can the vitamin A be recovered from them. He has recently prepared a crystalline vitamin A *beta*-naphthoate, $C_{31}H_{36}O_2$, m.p. 76° , from a vitamin A concentrate and *beta*-naphthoyl-chloride, in the presence of pyridine. This naphthoate exhibited a vitamin A activity of 2700 CLO and, when saponified by a 10 per cent potassium hydroxide solution, yielded vitamin A and *beta*-naphthoic acid.

Hamano has also obtained a vitamin A *anthraquinone beta*-carboxylate, $C_{20}H_{29}OCOC_{14}H_7O_2$, yellow hexagonal plates (from acetone), m.p. 124° , with a vitamin A activity of about 6000 CLO.

Other Approaches to the Synthesis of Vitamin A. The synthesis of pure vitamin A has not as yet been achieved, although numerous attempts have been and are still being made to accomplish this, following the publication, in December, 1931, of the structure assigned to it by Karrer, Morf, and Schöpp.²²²

One of the first attacks upon the problem of its synthesis was that of Karrer, Salomon, Morf, and Walker,²²³ which may be presented schematically thus:



Squalene

²²¹ Hamano, *ibid.*, **23**, 69 (1935).

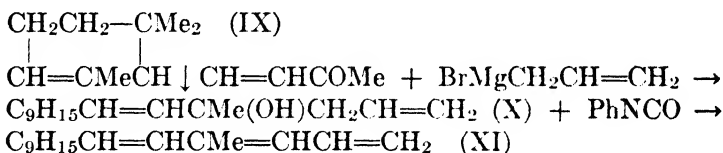
²²² Karrer, Morf, and Schöpp, *Helv. Chim. Acta*, **14**, 1434 (1931).

²²³ Karrer, Salomon, Morf, and Walker, *ibid.*, **15**, 878 (1932).

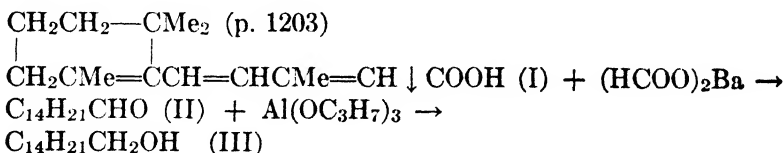
In this series of reactions, when an attempt was made to use the magnesium derivative of the bromide (V) with chloromethyl ether, so as to secure the ether (VI) for further lengthening of the side chain, only a little of this product was formed, together with some hydrocarbon, apparently by loss of hydrogen bromide from the original bromide (V). One of the main products of the reaction was a bimolecular condensation, resulting in a methylated dicyclohexyldecane (VII), which may be regarded as a perhydosqualene (VIII) cyclized at both ends, like the carotenes.

Another trail blazed by Karrer toward the same goal began with the condensation of allylmagnesium bromide with the ionones. In this way, *alpha*-ionone (IX) yielded the unsaturated alcohol (X), from which water was split out, when it was heated with phenyl isocyanate, giving the hydrocarbon (XI). The constitution of the latter was proved by the facts that on ozonolysis, it yielded 23.4 per cent of the calculated amount of isogeronic acid; by oxidation with chromic oxide, 2 molecules of acetic acid; and with permanganate, which attacks only the methyl group of the side chain, one molecule of acetic acid. In chloroform solution, it gave with antimony trichloride a deep brownish-red solution of violet tinge.

Strangely enough *beta*-ionone, subjected to the same reaction with allylmagnesium bromide, did not behave similarly. Little or no alcohol was formed, and it appeared that the bromide had added to one of the ethylene bonds.

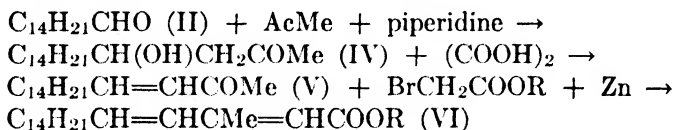


In England, Heilbron and his associates⁷ distilled at low pressure the barium salt of (I) with barium formate, and obtained the corresponding aldehyde (II), which was then reduced to the corresponding alcohol (III) by the action of aluminum isopropoxide. This alcohol (III) was devoid of any vitamin A activity.

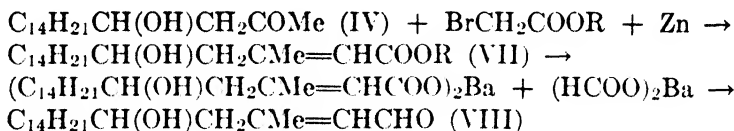


The aldehyde (II) was condensed with acetone, in the presence of

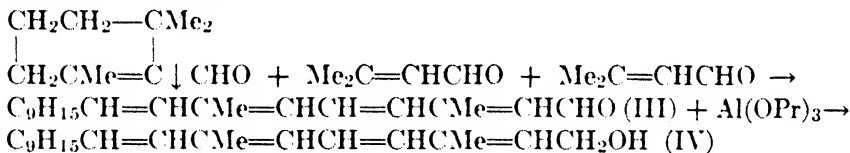
piperidine, the resultant alcohol (IV) dehydrated with anhydrous oxalic acid, and the unsaturated ketone (V) subjected to the Reformatsky reaction (p. 461) with ethyl bromoacetate, yielding an unsaturated ester (VI), which it is hoped to reduce to the corresponding aldehyde in the same way as (II) was obtained from (I).



Further, the ketol (IV), when treated with bromoacetic ester and zinc, gave the unsaturated ester (VII), from which the corresponding aldehyde (VIII) was secured by distilling the barium salt of the acid with barium formate, but this has not yet been dehydrated to the octatetraene.



Recently, Fuson and Christ²²⁴ have reported an attempt to prepare vitamin A (IV) by the condensation of one mole of *beta*-cyclocitral (I) with two of dimethylacrolein (*beta*-methylcrotonaldehyde) (II), followed by reduction of the supposititious aldehydic product (III) with aluminum isopropoxide:



The resulting solution gave a blue color with antimony trichloride in chloroform, and an ultra-violet absorption spectrum with a maximum in the vicinity of 328 m μ .

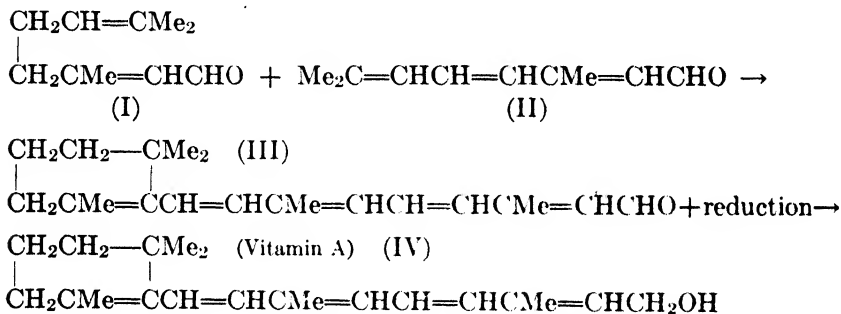
Heilbron and Jones,²²⁵ in commenting upon the above, have pointed out that antimony trichloride color reactions are much less reliable than spectroscopic evidence in such problems, and that the biological test is the most important one. Thus, the farnesinol of Fischer and Hultzsch (p. 1207) gives an intense blue with antimony trichloride in chloroform solution, and very probably has an absorption maximum in the neighborhood of 328 m μ .

²²⁴ Fuson and Christ, *Science*, **84**, 294 (1936).

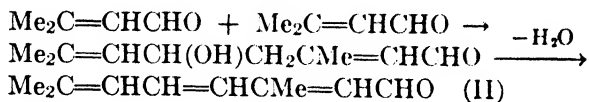
²²⁵ Heilbron and Jones, *Chemistry & Industry*, 813 (1936).

In their own experiments,^{225,226} attempts to condense *beta*-citral with crotonic or other aldehydes were unsuccessful, using the piperidine acetate catalyst of Kuhn, Badstübner, and Grundmann.²²⁷ Citral, however, condensed readily with one mole of crotonaldehyde, or its *beta*-methyl derivative, and the products were easily cyclized by a new method (not described). They are of the opinion, therefore, that citral offers a simpler approach than *beta*-ionone to the synthesis of vitamin A.^{227a}

At Prague, Czechoslovakia, Bernhauer and his collaborators^{228,229,230,231,232} have been trying for several years to reach the vitamin A goal by a somewhat similar route, namely, by condensing citral (I) with 2,6-dimethyloctatrienal-8 (II), followed by cyclization (III) and then reduction of this aldehyde to the corresponding alcohol (vitamin A) (IV)



It was hoped to obtain the necessary trienal (II) by condensation of two moles of *beta*-methylcrotonaldehyde,



but in this they have not as yet been successful.

In connection with these investigations, they reported also,²³² some three months prior to the above article by Heilbron and Jones²²⁵ in the same field, that crotonaldehyde condensed with citral, in the presence of barium oxide, with formation of a crystalline product, $\text{C}_{14}\text{H}_{20}\text{O}_2$, m.p. 99°, of undetermined structure.

²²⁶ Heilbron, Jones, Lowe, and Wright, *J. Chem. Soc.*, 561 (1936).

²²⁷ Kuhn, Badstübner, and Grundmann, *Ber.*, **69**, 98 (1936).

^{227a} See, also, Batty, Burawoy, Heilbron, Jones, and Lowe, *J. Chem. Soc.*, 755 (1937).

²²⁸ Bernhauer and Irrgang, *Biochem. Z.*, **254**, 434 (1932).

²²⁹ Bernhauer and Neubauer, *ibid.*, **251**, 173 (1932).

²³⁰ Bernhauer and Woldan, *ibid.*, **249**, 199 (1932).

²³¹ Bernhauer and Drobnick, *ibid.*, **266**, 197 (1933).

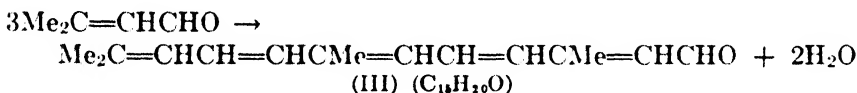
²³² Bernhauer, Irrgang, Adler, Mattauach, Müller, and Neiser, *Ann.*, **525**, 43 (1936).

Wittig and his co-laborers,²³³ recalling the fact that the classical method of synthesizing polyene aldehydes, $R(\text{CH}=\text{CH})_n\text{CHO}$, is by repeated condensations with acetaldehyde, $R\text{CHO} + \text{CH}_3\text{CHO} \rightarrow R\text{CH}=\text{CHCHO} + \text{CH}_3\text{CHO} \rightarrow R(\text{CH}=\text{CH})_2\text{CHO}$, etc., bring out forcibly the objections to the method because the $R\text{CHO}$ reacts not only with the acetaldehyde, but also with itself, resulting in mixtures which are often difficult to separate, and yields therefore are likely to be low.

The condensation of RCHO with CH_3COCOOH , to compounds of the type $\text{RCH}=\text{CHCOCOOH}$, as carried out by Fischer and Wiedemann¹⁷⁶ (p. 1212), followed by the elimination of carbon dioxide, has not proved satisfactory, probably on account of the instability of the aldehyde so formed.

The authors therefore have had recourse to the following series of reactions: $\text{RCHO} + \text{H}_2\text{C}(\text{CN})\text{COOH} \rightarrow \text{RCH}=\text{C}(\text{CN})\text{COOH} + \text{Cu} \rightarrow \text{RCH}=\text{CHCN} + \text{HCl} \rightarrow \text{RCH}=\text{CHCCl}=\text{NH} + \text{SnCl}_2 \rightarrow \text{RCH}=\text{CHCH}=\text{NH} + \text{H}_2\text{O} \rightarrow \text{RCH}=\text{CHCHO}$, and in this way these colorless aldehydes were obtained: $\text{PhCH}=\text{CHCH}=\text{CHCHO}$ and $\text{Ph}_2\text{C}=\text{CHCH}=\text{CHCHO}$.

Fischer and Hultzs^{23,4} have also studied the condensation of *beta*-methylcrotonaldehyde (I) and found that, with weak aqueous alkali, it yielded dimethyloctatrienal (II) (dehydrocitral) (lemon yellow), farnesinal (III) (golden yellow), and other products.



Of the crude reaction product, 50 per cent could be distilled at low pressure, and of this distillate about one-half was (II) and one-fifth (III). The dehydrocitral (II) could not be recovered as such, but could be oxidized to the corresponding (dehydrogeranic) acid by silver oxide, or reduced to the alcohol (dehydrogeraniol) with aluminum isopropoxide. The farnesinal (III) was similarly reduced to farnesinol; or, on catalytic hydrogenation, it added ten atoms of hydrogen, with formation of hexahydrofarnesal.

Fischer, Hultsch, and Flaig^{23,4a} have also synthesized from crotonaldehyde, dodecapentenol, dodecapentenol and tetradecahexadienoic acid.

²³³ Wittig, Kethur, Klein, and Wiethrock, *Ber.*, **69**, 2078 (1936).

²³⁴ Fischer and Hultsch, *Ber.*, **68**, 1726 (1935).

^{234a} Fischer, Hultsch, and Flaig, *Ber.*, **70**, 370 (1937).

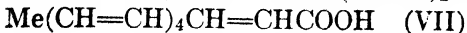
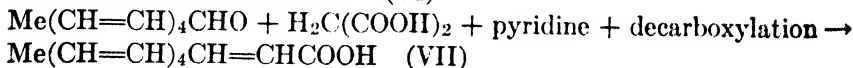
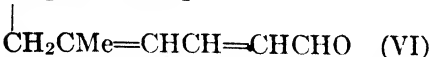
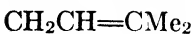
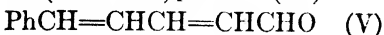
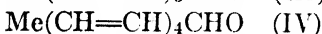
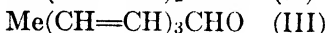
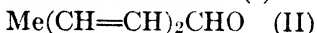
All these new polyene aldehydes and alcohols, especially the lower-melting ones, were extraordinarily sensitive to oxygen. Farnesinol, in chloroform solution with antimony trichloride, gave an inky blue color, resembling that produced by vitamin A under similar conditions.

In comparison with citral and geraniol, the odors of dehydrocitral and dehydrogeraniol were weaker, sweeter, more floral, and (especially the aldehyde) less spicy and refreshing. Those of farnesinal and farnesinol were very faint, that of the alcohol being the sweeter and more floral.

According to the experience of Kuhn, Badstübner, and Grundmann,²²⁷ pure crotonic aldehyde (I) cannot be condensed to octatrienal (III) by piperidine, except after exposure to the light of the sun (or of a quartz lamp), or by the addition of acid catalysts. In other words, the actual catalyst appears to be the piperidine salt, and not piperidine itself. Thus, piperidine acetate has proved very satisfactory as a catalyst for the condensation of a number of these aldehydes. Among the products thus obtained from the interaction of (I) and acetaldehyde, was a small quantity of decatetraenal (IV), m.p. 107–107.5°, which gave a dark wine red with concentrated sulfuric acid.

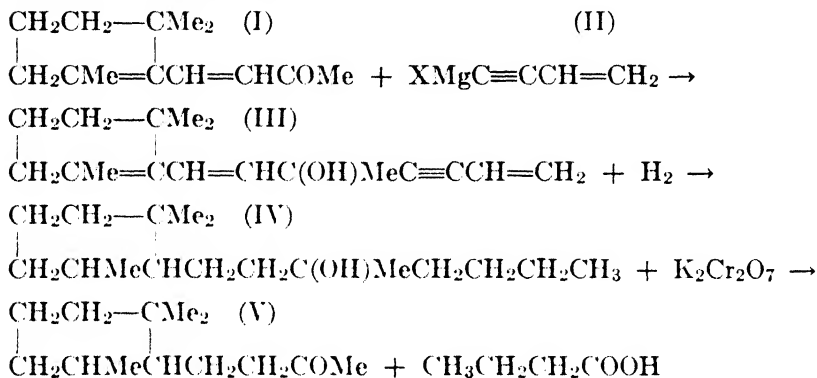
Benzaldehyde was condensed similarly with crotonic aldehyde (I), to form phenylpentadienal (V), and citral with acetaldehyde, to give citrylidene acetaldehyde (VI). The authors noted that the odor and physical constants of the latter product differed somewhat from those given by von Braun and Rudolph²³⁵ for their citrylidene acetaldehyde, and also that reduction with aluminum isopropoxide to the citrylidene ethyl alcohol yielded a product whose rose odor was more persistent than that of geraniol.

When the decatetraenal (IV) was condensed with malonic acid, in the presence of pyridine, and the dicarboxylic acid heated, there resulted a dodecapentaenoic acid (VII), which melted with decomposition at 247°, and gave a brownish red color with concentrated sulfuric acid.



²³⁵ v. Braun and Rudolph, *Ber.*, **67**, 1735 (1934).

Another approach to the synthesis of compounds related to vitamin A, or the semi-carotenes, was that of Zal'kind, Zonis, and Blokhin,²³⁶ who condensed *beta*-ionone (I) with the magnesium derivative of vinyl-acetylene (II), and reduced the product (III) catalytically to the saturated tertiary alcohol (IV), whose constitution was established by its oxidation to tetrahydroionone and butyric acid.



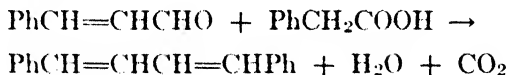
Two Russian investigators, Teterin and Ivanov,²³⁷ investigated the action of magnesium upon a mixture of *beta*-ionone and 1,4-dibromo-2-butene, and found that it resulted only in the reduction of the ionone to the corresponding pinacol.

Synthesis of Other Polyenes

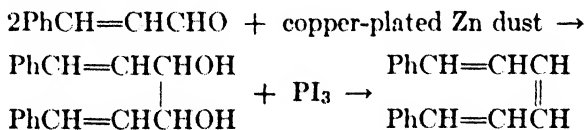
Because of their fundamental importance in the development of the present theories concerning the connection between color and constitution in the polyene pigments, the syntheses of certain diphenylpolyenes, and polyene carboxylic acids, are given here.

Synthesis of diphenylpolyenes¹⁷⁵ (p. 1179).

(a) $\text{Ph}(\text{CH}=\text{CH})_2\text{Ph.}$ —

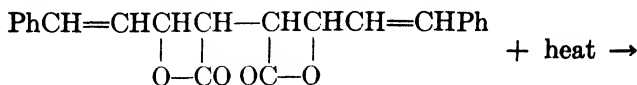
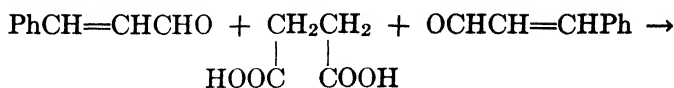


(b) $\text{Ph}(\text{CH}=\text{CH})_3\text{Ph.}$ —

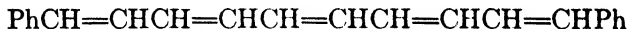
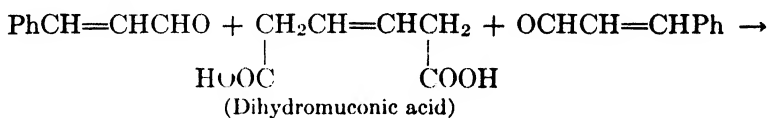
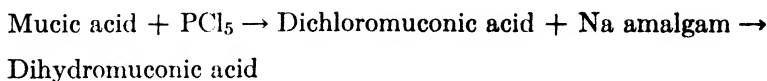
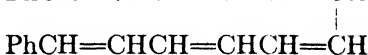
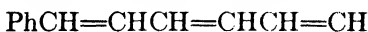
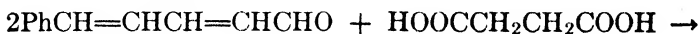
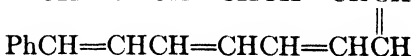
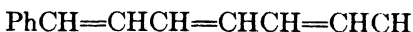
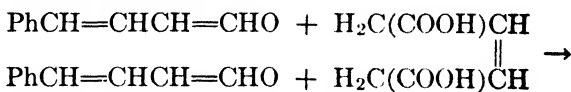
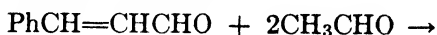


²³⁶ Zal'kind, Zonis, and Blokhin, *Compt. rend. acad. sci. U.R.S.S.*, **2**, 57 (in German 61) (1935).

²³⁷ Teterin and Ivanov, *ibid.*, **2**, 259 (in German 260) (1935).

(c) $\text{Ph}(\text{CH}=\text{CH})_4\text{Ph}.$ —

This diphenyloctatetraene is the easiest one of the colored series to prepare, the yield from a kilo of succinic acid being as high as 700 grams.

(d) $\text{Ph}(\text{CH}=\text{CH})_5\text{Ph}.$ —(e) $\text{Ph}(\text{CH}=\text{CH})_6\text{Ph}.$ —(f) $\text{Ph}(\text{CH}=\text{CH})_7\text{Ph}.$ —(g) $\text{Ph}(\text{CH}=\text{CH})_8\text{Ph}.$ —

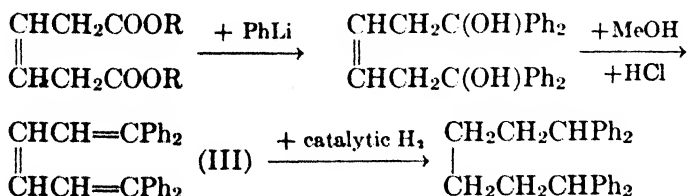
Wittig and Klein¹⁷⁹ have conducted some interesting studies on the connection between color and constitution in the following tetraphenyl, tetrabiphenyl, and related polyene hydrocarbons:

- (I) $\text{Ph}_2\text{C}=\text{CPh}_2$ (Diphenylstilbene), colorless.
- (II) $\text{Ph}_2\text{C}=\text{CHCH}=\text{CPh}_2$ (1,1,4,4-Tetraphenylbutadiene), colorless.
- (III) $\text{Ph}_2\text{C}(=\text{CHCH}=\text{C})_2\text{CPh}_2$ (1,1,6,6-Tetraphenylhexatriene), pale yellow.
- (IV) $\text{Ph}_2\text{C}(=\text{CHCH}=\text{C})_3\text{CPh}_2$ (1,1,8,8-Tetraphenyloctatetraene), yellow.
- (V) $\text{Ph}_2\text{C}(=\text{CHCH}=\text{C})_4\text{CPh}_2$ (1,1,10,10-Tetraphenyldecapentaene), orange.
- (VI) $\text{Ph}_2\text{C}=\text{C}_6\text{H}_4=\text{CPh}_2$ (Tetraphenyl-*p*-xylylene), orange.
- (VII) $\text{Ph}_2\text{C}=\text{C}_{10}\text{H}_6=\text{CPh}_2$ (Naphthoquinone-2,6-*bis* [diphenylmethide]), red.
- (VIII) $\text{Ph}_2\text{C}=\text{C}_6\text{H}_4=\text{C}_6\text{H}_4=\text{CPh}_2$ (Diphenoquinone-*p,p'*-*bis*[diphenylmethide]), violet.
- (IX) $\text{Ph}_2\text{C}=\text{C}_6\text{H}_4=\text{CHCH}=\text{C}_6\text{H}_4=\text{CPh}_2$ (*p,p'*-*bis* [diphenylmethenyl] stilbene), blue-violet.
- (X) $(\text{PhC}_6\text{H}_4)_2\text{C}=\text{CHCH}=\text{CHCH}=\text{C}(\text{C}_6\text{H}_4\text{Ph})_2$ (1,1,6,6-Tetrabiphenylhexatriene), deep egg yellow.

Compounds (VI) to (IX) inclusive constitute a series in which increase in the length of the polyene chain between the Ph_2C groups results in a shift of the light absorption toward the longer wave lengths and a great increase in sensitivity to oxygen. This deepening of color is postulated as due to a greater loosening of the valence electrons.

Compounds (I) to (V) inclusive were prepared, therefore, for an analogous comparison, and similar color changes were observed, together with a greatly increased stability to oxygen.

Of these compounds, (III) was synthesized as follows:

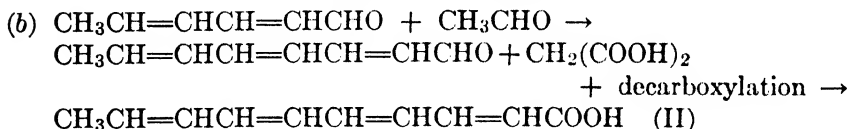
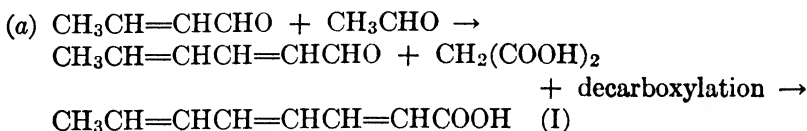


The analogous tetrabiphenyl derivative (X), prepared similarly,

possessed a deeper egg yellow color and a stronger greenish yellow fluorescence than (III) and, like it, was indifferent to oxygen.

Pursuing the Kuhn and Winterstein process (p. 1210), compound (IV) was synthesized from $\text{Ph}_2\text{C}=\text{CHCHO}$, succinic anhydride, lead oxide, and acetic anhydride; and, by replacing the succinic anhydride with dihydromuconic acid, compound (V) was similarly prepared.

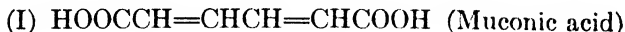
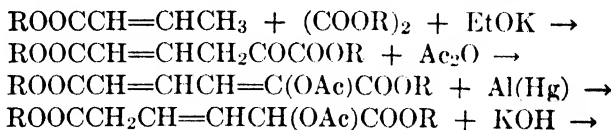
Synthesis of Polyene Carboxylic Acids. Octatrienic (I), and decatetraenic (II) acids, have been synthesized by Kuhn and Hoffer,^{238,239} by the following steps:



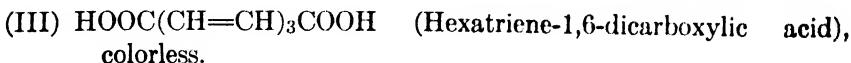
By condensing polyene aldehydes with pyruvic acid, Fischer and Wiedemann¹⁷⁶ obtained the corresponding *alpha*-keto polyene acids.

$\text{CH}_3(\text{CH}=\text{CH})_n\text{CHO} + \text{CH}_3\text{COCOOH} \rightarrow \text{CH}_3(\text{CH}=\text{CH})_n\text{COCOOH}$
When n was 1, the product was a bright yellow; with $n = 2$, it was an indian yellow; and with $n = 3$, it was orange red. By oxidation with silver oxide, these keto acids were converted into the corresponding polyene carboxylic acids, $\text{CH}_3(\text{CH}=\text{CH})_n\text{COOH}$.

The polyene *alpha,omega*-dibasic acids have been prepared by Kuhn and Grundmann,^{177, 178} through these steps:



In a similar manner, from ethyl oxalate and the appropriate $\text{Me}(\text{CH}=\text{CH})_n\text{COOR}$, the following acids were secured:



²³⁸ Kuhn and Hoffer, *Ber.*, **63**, 2164 (1930).

²³⁹ Kuhn and Hoffer, *Ber.*, **65**, 651 (1932).

(IV) $\text{HOOCCH}=\text{CHCH}=\text{CMeCH}=\text{CHCOOH}$ (3-Methylhexatriene-1,6-dicarboxylic acid).

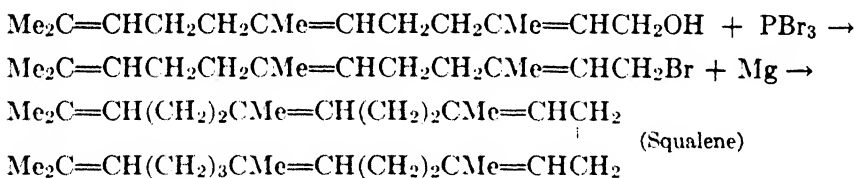
(V) $\text{HOOC}(\text{CH}=\text{CH})_4\text{COOH}$ (Octatetraene-1,8-dicarboxylic acid), chrome yellow.

(VI) $\text{HOOC}(\text{CH}=\text{CH})_5\text{COOH}$ (Decapentaene-1,10-dicarboxylic acid), orange yellow.

When (III) was reduced with sodium amalgam, a 1,6 addition occurred, with production of the colorless acid $\text{HOOCCH}_2(\text{CH}=\text{CH})_2\text{CH}_2\text{COOH}$, whose structure was established by condensing it with two moles of $\text{PhCH}=\text{CHCHO}$ to $\text{Ph}(\text{CH}=\text{CH})_6\text{Ph}$ (p. 1210). The chrome yellow (V), subjected to a like reduction, yielded the analogous colorless acid, $\text{HOOCCH}_2(\text{CH}=\text{CH})_3\text{CH}_2\text{COOH}$.

Synthesis of Squalene and of Phytol. Since both squalene^{240, 241, 242, 243} and phytol are of importance in a study of the relation of the carotenoids to other classes of compounds of biochemical interest, the syntheses by which their constitutions were established are given briefly here.

(a) *Squalene* was prepared by Karrer and Helfenstein²⁴⁴ by the action of metallic potassium or magnesium upon farnesyl bromide:



(b) *Phytol*. Fischer and Löwenberg^{245, 246} confirmed the structural formula proposed for phytol by its synthesis from hexahydropseudoionone as initial material. In the following formulas, that portion of the hexahydropseudoionone to the left of the vertical arrow is represented by R.

²⁴⁰ U. S. pat. 1,961,683, Bunbury, Sexton, and Stewart (to Imperial Chemical Industries, Ltd.) (June 5, 1934); [*C. A.*, **28**, 4929 (1934)].

²⁴¹ Brit. pat. 345,734, Imperial Chemical Industries, Ltd. (Jan. 22, 1930); [*C. A.*, **26**, 1944 (1932)].

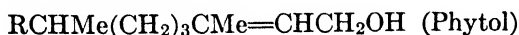
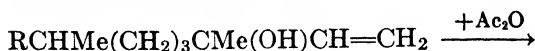
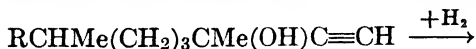
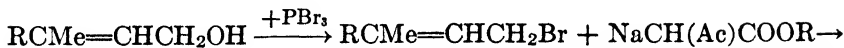
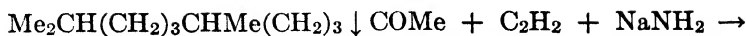
²⁴² Fr. pat. 709,862, Imperial Chemical Industries, Ltd. (Jan. 22, 1931); [*C. A.*, **26**, 995 (1932)]. Dye. Halogen derivatives of squalene + phenols + AlCl_3 = phenols which can be used as couplers for azo dyes.

²⁴³ Ger. pat. 539,271, Imperial Chemical Industries, Ltd., Bunbury and Sexton (Jan. 21, 1931); [*C. A.*, **26**, 1944 (1932)].

²⁴⁴ Karrer and Helfenstein, *Helv. Chim. Acta*, **14**, 78 (1931).

²⁴⁵ Fischer and Löwenberg, *Ann.*, **464**, 69 (1928).

²⁴⁶ Fischer and Löwenberg, *Ann.*, **475**, 183 (1929).



THE RELATION OF CAROTENOIDS TO OTHER CLASSES OF COMPOUNDS

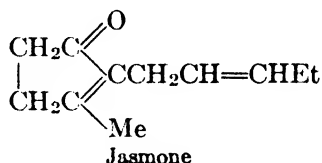
Relation to the Terpenes (p. 6). Like the terpenes, the carotenoids are generally regarded as built up of isoprene (C_5H_8) units, for the most widely distributed members of each class possess molecular formulas in which the carbon is some simple multiple of 5. Thus, there are the hemiterpenes (C_5H_8), simple terpenes ($\text{C}_{10}\text{H}_{16}$), sesquiterpenes ($\text{C}_{15}\text{H}_{24}$), diterpenes ($\text{C}_{20}\text{H}_{32}$), etc., in the terpene class; and, in the carotenoids, the C_{20} (crocetin), C_{25} (bixin), and C_{40} (carotenes, xanthophylls, etc.) groups.

The common carotenoids of the C_{40} group may be looked upon as tetraterpenes so extensively dehydrogenated, with formation of long chains of chromophoric conjugated double bonds, that their absorption bands have been pushed far over into the visible region of the spectrum.

One of the most direct, and certainly one of the most interesting, lines of connection between the two groups, is that between the ionones and the carotenes. It has been known for many years that carotene, on long standing in the air, exhales a strong violet odor, and that its oxidation products (p. 1193) are similar to those obtained from ionone. Further, evidence has already been given (p. 1164) that lycopene is an aliphatic $\text{C}_{40}\text{H}_{56}$ carotenoid, that in *gamma*-carotene one end of the chain is closed to an ionone cycle, and that in *alpha*- and *beta*-carotene both ends are similarly cyclized to either an *alpha*- or a *beta*-ionone nucleus. The close analogy to the behavior of pseudoionone, *alpha*-, and *beta*-ionones, is immediately apparent. Just as pseudoionone closes to both *alpha*- and *beta*-ionones, so natural carotene is usually a mixture of both *alpha*- and *beta*-carotene. A common occurrence in the terpene field, particularly under biochemical influences, is the opening and

closing of the cycle with formation of various isomers, which are therefore often found closely associated in nature.

As Kuhn and Brockmann observe,⁵ the ease with which the cyclopentene ring is formed from 1,6-diketones by the action of alkalies, and the readiness with which cyclization takes place even in the carotenoid group, suggests the probability that jasmone



is produced in the plant similarly from a 1,4-diketone, in which the methylene group in position 5 brings about the condensation.

The sesquiterpene alcohol, farnesol, $\text{C}_{15}\text{H}_{25}\text{OH}$, one of the beautiful perfume constituents of orange, acacia, and cananga flowers, as well as of ambrette seed, carries a terminal group which bears a striking structural resemblance to that present in squalene and in lycopene. These connections will appear on examining the graphic formulas as given on pp. 1154 and 1213.

Reverting to one of the characteristics of the carotenoids, viz., the so-called reversal of the isoprene sequence in the middle of the principal chain, with formation of a $-\text{CMe}=\text{CHCH}=\text{CHCH}=\text{CMe}-$ group, it may be pointed out that this is not peculiar to the polyene pigments, as witnessed by the existence of this same group in squalene, $\text{C}_{30}\text{H}_{50}$, a triterpene of shark-liver oil.

A glance at this squalene formula will show also that, by the removal of one H from each of the CH_2 groups, a polyene pigment should result carrying a sequence of 11 conjugated double bonds.

Relation to Vitamins A and C. The close connection between *beta*-carotene and vitamin A has been discussed on pp. 1167 and 1198.

So far as vitamin C (p. 1503) is concerned, it has been reported by Giroud and his collaborators²⁴⁷ that fruits containing carotenoid pigments are rich in vitamin C, while those otherwise colored contain but little of it; that vitamin C generally accompanies chlorophyll; and that, in the ripening of certain fruits, as the chlorophyll disappears, the carotenoid and vitamin C increase.

Relation to the Sterols (p. 1262). The connection between the carotenoids and the sterols is still obscure, but the two are commonly found

²⁴⁷ Giroud, Ratsimamanga, Leblond, Chalopin, and Rabinowics, *Bull. soc. chim. biol.*, **18**, 573 (1936); Willstaedt, *Skand. Arch. Physiol.*, **75**, 155 (1936).

associated in many natural products,²⁴⁸ especially in the unsaponifiable portion of fats, and most of the polyene pigments, as well as the sterols, are characterized by the presence of hydroaromatic cycles (ionones or hydrophenanthrenes).

Of the various attempts which have been made^{249, 250, 251, 252, 253} to show graphically how cholesterol might be formed from polyterpenoid hydrocarbons, two are depicted on p. 1217.

Relation to Waxes, Fats, and Lipids. In discussing the occurrence in nature of the carotenoids, it was mentioned that they are found either free or combined (as esters, glycosides (p. 1454), or chromoprotids), and usually in colloidal solution in the lipids.

The carotenoid alcohols thus function in much the same way as other alcohols in the formation of fats and waxes. Like common fats which can be technically hardened, these pigment waxes can be catalytically hydrogenated smoothly to colorless products which closely resemble ordinary fats and waxes.

The living cell thus builds up (1) ordinary fats and waxes, sterol esters, lecithins, etc., from colorless alcohols and colorless acids; (2) carotenoids, chlorophyll, etc., from colored (polyene) acids (crocetin, etc.) and colorless alcohols (methanol, phytol, etc.); and (3) carotenoid pigment waxes from colored polyene alcohols and colorless acids. Further, it is quite probable that there will be discovered also in nature some pigment waxes in which polyene alcohols are esterified with polyene acids for the biogenetic processes of nature often result in the esterification of an alcohol with its corresponding acid.

It is interesting to note that, in these biosyntheses, the same aliphatic acid may be esterified either with a saturated alcohol to form a colorless wax, or with a polyene alcohol to form a pigment wax. Where the two are found associated in the same plant, their genetic relation is still obscure.

Relation to Albuminates. Whereas in the plant kingdom the carotenoids are combined usually as esters and occasionally as glycosides, in the animal kingdom they are often found united with albumin, particularly in the Crustacea and other marine animals, where it has been suggested that the polyene may play a role resembling that of the hemin in hemoglobin. By acid hydrolysis, the carotenoid can be set free from

²⁴⁸ Basu, *Biochem. Z.*, **274**, 4 (1934).

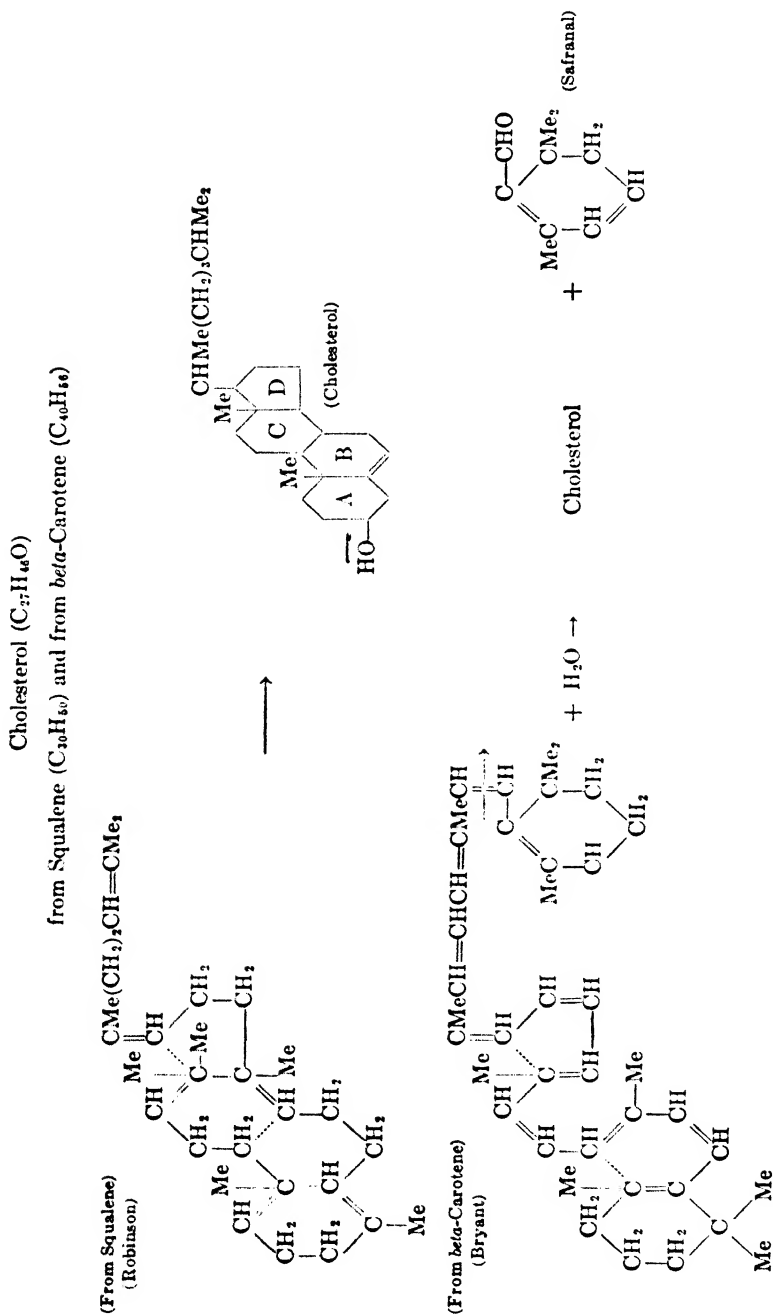
²⁴⁹ Vangelovici, *J. Soc. Chem. Ind.*, **53**, 998 (1934).

²⁵⁰ Robinson, *ibid.*, **53**, 1062 (1934).

²⁵¹ Bryant, *Chemistry & Industry*, **54**, 907 (1935).

²⁵² Bryant, *ibid.*, **54**, 1082 (1935).

²⁵³ Spring, *J. Soc. Chem. Ind.*, **54**, 973 (1935).



such combinations. The physiological function of these compounds in the animal organism is still unknown.

Relation to Flavins. Another class of natural pigments which, like the carotenoids, appear to be essential to animal life, are the so-called "flavins," or "lyochromes," of which the lactoflavin appears to be identical with vitamin B₂ (p. 1013).

Kuhn has called attention to the interesting way in which the properties of these two classes of pigments are mutually complementary:

	CAROTENOID	FLAVIN
Composition.....	Non-nitrogenous	Nitrogenous
In water.....	Insoluble	Soluble
To acids.....	Unstable	Stable
To alkalis.....	Stable	Unstable
To oxidizing agents.....	Unstable	Stable
Related to.....	Vitamin A	Vitamin B ₂

Further, the effective daily dose, as measured by the growth of rats, is the same (5 *gamma*) for lactoflavin as for carotene.

Whether or not these two classes are biogenetically related in some way has not been discovered.

GENERAL REFERENCES

The literature has been examined up to August 1, 1937, at least so far as it is reported in *Chemical Abstracts*. The references are grouped under the two headings: A. Books; B. Reviews, Lectures, etc. The arrangement under A and B is first chronological, then alphabetical according to the name of the author first mentioned. Of the 2000 or more general and specific references found in the literature, only a few are included, because of space limitations. The literature up to the middle of 1922 is very fully covered in Palmer's classic work (in A), and from then to the middle of 1934 by Zechmeister's masterly treatise (in A). The citations in the text and those which follow, therefore, relate mostly to work which has appeared since.

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CHAPTER 15

THE STEROLS, BILE ACIDS, AND RELATED COMPOUNDS

(The Cyclopentanoperhydrophenanthrene or Steroid Group)

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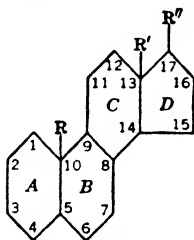
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THE CYCLOPENTANOPERHYDROPHENANTHRENE OR STEROID GROUP *

Introduction

Among the substances found in nature is a family of compounds derived from the hydrocarbon cyclopentanoperhydrophenanthrene. All the natural compounds are oxygenated, alkyl-substituted derivatives of this parent hydrocarbon, and have the ring system given in structure I.† The recognized members of the group are the sterols, the bile acids, the aglucons of the cardiac glycosides, the genins of the toad poisons,



I Ring system of the steroid group.
(R, R' and R'' indicate aliphatic side chains.)

* Of these two designations, "cyclopentanoperhydrophenanthrene" is the more precise and "steroid" the more convenient. The term "steroid" (like the sterols) was suggested in 1936 by Callow and is being widely adopted. Prior to this suggestion, Rosenheim had proposed that the group be named the "cholane" group but this term never gained wide usage. The chief objection against "cholane" as a group name is that cholane is the hydrocarbon obtained from cholanolic acid (XLIII, p. 1244) by replacement of the carboxyl with methyl, and thus all the compounds are referred to a hydrocarbon in which the C₁₇ side chain is intermediate in length.

In the preparation of this review, the literature up to September, 1937, has been considered. The growth of the field is so rapid, however, that any review is soon somewhat out-of-date. It may be noted here that the entire field has been admirably reviewed in monographs by Fieser, and by Lettré and Inhoffen, both of which were published early in 1936. Fieser later published an appendix to his monograph and summarized the work that appeared in 1936. Citations from these books will be made by giving the author's name followed by the page or chapter reference. These and other monographs are reviewed at the end of this chapter.

† The method of numbering the ring system and the substituent side chains is illustrated in formulas I and II. The system of numbering is irrational and was adopted to permit an easy transition from the old formulations, illustrated in IIa and IIIa, to the new structures.

the saponinins of the digitalis saponins, the sex hormones, certain adrenal substances, and a few other compounds which are not easily classified. The outstanding chemical characteristics of the several members, together with their principal sources, are summarized in Table I.

Variations within the group include changes (1) in the nature of the side chains, R, R', R''; (2) in the spatial configuration of the nucleus and its substituent groups; (3) in the number and position of the hydroxyl groups; and (4) in the degree and position of unsaturation. As is shown in Table I, the nature of the side chain, R'', changes markedly, while R' is always a methyl group, and R, when present, represents either a methyl group, an oxidation product of one, or hydrogen. Since the nucleus is alicyclic, stereoisomeric modifications (p. 244) of the type exhibited by the decalins occur. Actually the mutations in nuclear spatial configuration are few, since the relationship of rings B/C and C/D appears to be the stable *trans* arrangement in most of the known compounds. The relationship of rings A/B is variable, and, through usage, those compounds which have the *trans* configuration are designated as *allo*-structures. Although groups R and R' may be attached so that they project either into or out from the plane of the paper, they are usually assumed to be projecting out from the paper. Since these groups are generally methyl radicals and are attached at an angle to a carbon atom shared by two rings, they are referred to as the angular methyl groups.

Most of the members of the cyclopentanoperhydrophenanthrene group have an hydroxyl group attached at C₃, and many are oxygenated elsewhere. These hydroxyl groups may be attached either *cis* or *trans* to any given reference point.* There is some variance in the choice of point of reference, but in this chapter the spatial configuration will be referred to the nearest angular methyl group. To indicate the difference in configuration, a solid line will be used for groupings that may be regarded as projecting out from or lying in the plane of the paper, a dotted line for groups projecting into the plane of the paper. The difference in configuration of the C₃—OH group is described by a number of prefixes. In the sterol series, those C₃—OH groups that are different from the normal are designated as *epi*-configurations; in the bile acids one position is called the α -type, the other the β -type. On relating these designations, the normal and β -type are *cis*, while the *epi* and α -type are *trans*, to the reference point, C₁₀—CH₃.

Generalizations as to the position and degree of unsaturation cannot

* See note, p. 1256.

TABLE I
MEMBERS OF THE CYCLOPENTANOPEHYDROPHENANTHRENE GROUP

Member	Class of Compound	Side Chains Attached to Ring System (I)			Sources
		R	R'	R''	
STEROLS.....	Saturated and unsaturated secondary alcohols	$-\text{CH}_3$	$-\text{CH}_3$	$\begin{array}{c} \text{CH}_3 \\ \\ -\text{CH}_2- \end{array}$ or $\begin{array}{c} \text{CH}_3 \\ \\ -\text{CH}-(\text{CH}_2)_3- \end{array}$ or substituted isooctyl with or without unsaturation	Tissues of animals, plants, and fungi
BILE ACIDS.....	Hydroxy acids	$-\text{CH}_3$	$-\text{CH}_3$	$\begin{array}{c} \text{CH}_3 \\ \\ -\text{CH}-\text{CH}_2-\text{CH}_2-\text{CO}_2\text{H} \\ \text{(generally)} \end{array}$	Bile
HEART POISONS Cardiac aglucons.....	Unsaturated hydroxy lactones	$-\text{CH}_3$, $-\text{CH}_2\text{OH}$ (?) or $-\text{CHO}$	$-\text{CH}_3$	$\begin{array}{c} \text{CH}_2-\text{C}=\text{O} \quad \text{CH}=\text{CH}-\text{C}=\text{O} \\ \quad \quad \\ -\text{C}=\text{HC} \quad \text{O} \quad \text{or} \quad -\text{C}=\text{CH}-\text{O} \end{array}$	Leaves, seeds, and roots of digitalis and related plants
Toad poisons.....	Unsaturated hydroxy and acetoxy lactones	$-\text{CH}_3$ (?)	$-\text{CH}_3$	$\begin{array}{c} \text{CH}=\text{CH}-\text{C}=\text{O} \\ \\ -\text{C}=\text{CH}-\text{O} \end{array} \quad (?)$	Parotid secretion of toads

TABLE I—Continued

Member	Class of Compound	Side Chains Attached to Ring System (I)			Sources
		R	R'	R''	
DIGITALIS SAPOGENINS.....	Hydroxy cyclic ethers	—CH ₃	—CH ₃	$ \begin{array}{c} \text{CH}_3 \\ \\ \text{—CH—CH—CH—CH}_2 \cdot \\ \quad \quad \\ \text{O} \quad \text{O} \quad \text{CH—CH}_3 \\ \diagup \quad \diagdown \\ \text{(C}_{10}\text{)} \quad \text{CH}_2 \end{array} $	Leaves, seeds, and roots of digitalis, etc.
SEX HORMONES					
Estrogenic.....	Phenolic alcohols or ketones	—CH ₃	=O or —OH	Gonads, placenta, urine
Corpus luteum.....	Unsaturated diketone	—CH ₃	—CH ₃	—CO—CH ₃	Corpus luteum
Androgenic.....	Saturated and unsaturated keto-alcohols or dihydric alcohols	—CH ₃	—CH ₃	=O or —OH	Gonads, urine
ADRENAL SUBSTANCES.....	Unsaturated ketonic alcohols	—CH ₃	—CH ₃	$ \begin{array}{c} \text{OH} \quad \quad \text{O} \\ \quad \quad \parallel \\ \text{—CH—CH}_2\text{OH, —C—CH}_2\text{OH, etc.} \end{array} $	Adrenal glands

* A furan ring is formed with the nucleus at C₁₀.

be made. The members of the group exhibit varying degrees of unsaturation, but in no instance, among the natural compounds, is the entire ring system aromatic.

The chemical study of any group of natural compounds passes through three stages: isolation, constitutional investigation, and synthesis. In the members of the cyclopentanoperhydrophenanthrene group, the last stage has not been realized. Serious handicaps often had to be overcome in the course of isolation, for where the substances were abundantly present in nature, mixtures of closely allied compounds were frequently encountered. Because of the size of the molecules, the usual methods of characterization did not always lead to satisfactory conclusions, and, in many instances, new physical and biochemical methods were necessary for precise definition. The hormones and the adrenal substances presented further difficulties, since only microscopic amounts were available for study. Because of these difficulties, microchemical methods have played a large part in the development of the chemistry of this group. Naturally, this need of microchemical methods stimulated their development. Pregl, for example, became interested in microanalysis through the study of the bile acids. In one extended oxidative degradation he obtained so little product that it seemed easier to develop methods for microanalysis than to repeat the transformation on a larger scale.¹

The sterols were the first of this group of compounds to be well investigated. Cholesterol, the principal sterol, has been known since 1788, when it was described by Poulletier.² The cardiac glycosides and the toad poisons are in a sense even older than the sterols, since their employment in medicine and as poisons extends into antiquity. The rational use of these cardiac principles in medicine, however, dates from 1785, the time of the publication of Withering's³ investigations on digitalis (foxglove). Chemical investigations began in 1841 with the isolation of a potent principle from digitalis;⁴ the work of importance started at the turn of the century. The bile acids were first isolated by Strecker⁵ in 1848, and, after some desultory work, have been actively investigated by a large number of workers since 1885. The digitalis saponins have been employed in crude form for centuries; the chemical work parallels that of the cardiac glycosides. The first sex hormone,

¹ Lieb, *Mikrochemie*, **1**, 63 (1923).

² Bills, *Physiol. Rev.*, **15**, 1 (1935). Actually cholesterol was probably discovered ca. 1769.

³ Withering, "An Account of the Foxglove and Some of its Medical Uses," Robinson, London (1785). Cf. Straub, *Münch. med. Wochschr.*, **65**, 888 (1935).

⁴ Homolle and Quevenne, *Arch. physiol. therap. Hygiene* (Bouchardat), Jan. 1854, p. 1.

⁵ Strecker, *Ann.*, **67**, 29 (1848).

estrone,* was isolated by Doisy in 1929. Since then the structures of most of the natural sex hormones have been established, and a number of "artificial" sex hormones have been prepared. The adrenal substances are not at the stage where established structures may be assigned to the several products obtained from adrenal extracts, but there are indications that this goal will soon be reached.

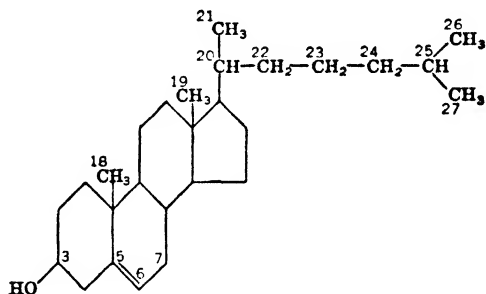
Although constitutional investigation began with the isolation of the various members of the group, the final phase of this work commenced in 1901 when Windaus (Göttingen) began his classical study of cholesterol, culminating in 1932 with a satisfactory structure for this important sterol. During this period and later, notable contributions to sterol chemistry were made by Diels (Kiel), Fernholz (Göttingen, later at Princeton in collaboration with Wallis), Heilbron (Manchester), Rosenheim (London), Schoenheimer (Columbia University), and a number of younger investigators at Göttingen—especially Lettré and Inhoffen. Other workers who have contributed to sterol and vitamin D chemistry are Anderson (Yale), Bergmann (Yale), Bills (Mead Johnson, Evansville), Callow (London), and Reindel (Wichenstephan-Munich). Paralleling and supplementing this work on the sterols were the investigations on the bile acids by Wieland (Munich), particularly with E. Dane, Borsche (Frankfort), Schenck (Leipzig), Windaus, and the Japanese investigators Shimizu and Yamasaki (Okayama). The chemistry of the cardiac aglucons has been developed by Jacobs (Rockefeller Institute), especially in collaboration with Elderfield, and to an equal degree by Tschesche (Göttingen). Much of the earlier work in this field was done by Kiliani (Freiburg) and Windaus, and certain special phases have been studied by Stoll (Sandoz, Basel). Wieland has been the chief worker in the field of the toad poisons, although within the last few years notable contributions have come from Chen (Eli Lilly, Indianapolis), Jensen (Johns Hopkins), Kondo and Ikawa (Tokyo), and Tschesche. The digitalis sapogenins have been studied by Tschesche, and Jacobs and Simpson, although the earlier work is due to Kiliani and Windaus. Noller (Stanford) has also made a number of contributions. In the field of the sex hormones, the work of Butenandt (Göttingen, Danzig, and Berlin) and Ruzicka (Zurich) predominates. Other important workers are Cook (London), Dirscherl (Boehringer, Mannheim-Waldhof), Doisy (St. Louis), Girard (Paris), Marker (Pennsylvania State College), Marrian (Toronto), and Wintersteiner (Columbia University), often in collaboration with Schwenk (Schering Corp., Bloomfield). Investigations leading to the isolation and characterization of the

* Estrone is frequently spelled "oestrone." In this discussion simplified spelling has been adopted wherever possible.

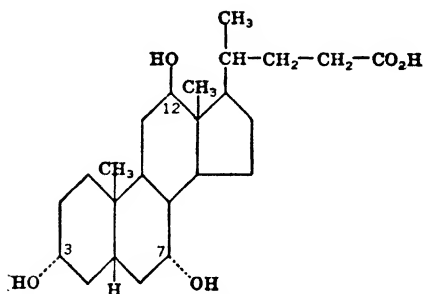
adrenal hormone cortin are in progress by Reichstein (Zurich), by Kendall and co-workers (Mayo Foundation), and by Wintersteiner and Pfiffner (Columbia University). Numerous other workers have participated in the development of the field; they will be mentioned in connection with their individual contributions.

Up to the year 1932 the structural investigations were concerned primarily with the nature of the nucleus. The study of this problem was carried out with the readily available cholesterol (II); with the common bile acids, cholic (III), desoxycholic, and α -hyodesoxycholic; and, to some extent, with the less common lithocholic and chenodesoxycholic acids. The early investigation of cholesterol, $C_{27}H_{46}O$, characterized it as a monohydric secondary alcohol, containing one double bond and an isoöctyl side chain. Similarly, the bile acids were recognized as hydroxy derivatives of cholanic acid, $C_{24}H_{40}O_2$, which, in turn, could be shown to contain the same nucleus as cholesterol, and a side chain,

$$\begin{array}{c} \text{CH}_3 \\ | \\ -\text{CH}-\text{CH}_2-\text{CH}_2-\text{CO}_2\text{H} \end{array}$$
 After allowing for the demands of the



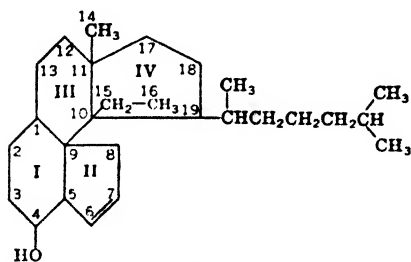
II Cholesterol
(C_3 -OH *cis* to C_{10} - CH_3)



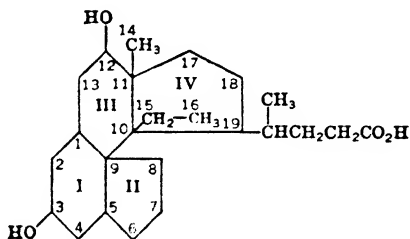
III Cholic acid
(3,7,12-Trihydroxycholanic acid)
 C_3 -OH and C_7 -OH *trans* to C_{10} - CH_3
Spatial position of C_{12} -OH uncertain

side chains, it was evident that the nucleus was hydroaromatic in nature, and apparently made up of four condensed rings. Owing to the lack of hetero atoms, the nature of the nucleus had to be examined by the methods of oxidative degradation and dehydrogenation.

In 1928 Windaus⁶ and Wieland⁷ reviewed in Nobel Prize addresses the results of their investigations on the sterols and the bile acids. The structures of cholesterol (IIa) and cholic acid (IIIa) which they discussed had been evolved by a study of the products of oxidative degradation, and seemed established in all details save for the attachment of carbon atoms 15 and 16. These were assumed to be present as an ethyl group at C₁₀. Subsequent attempts by Wieland⁸ to prove



IIa Old cholesterol structure



IIIa Old cholic acid structure

the position of the ethyl group led to the startling conclusion that there was no such grouping attached to ring IV of structures IIa and IIIa at the point in question. The two carbon atoms accordingly became "obdachlos" (homeless), and later investigation was concerned largely with attempts to place them in the ring nucleus. Wieland⁹ and Borsche¹⁰ both suggested structures in which the group $\text{CH}_3 - \text{CH} <$ was inserted in ring III, but the resulting seven-membered ring structures were never entirely acceptable.

A few years later Rosenheim and King¹¹ called attention to a neglected piece of evidence—the formation of chrysene as the product of dehydrogenation of cholesterol and cholic acid. On the basis of this fact, and of the x-ray measurements of ergosterol and calciferol by Bernal,¹² it was suggested that the ring nucleus of the sterols and the bile acids was perhydrochrysene. Study of the evidence in the light

⁶ Windaus, "Le Prix Nobel," Stockholm (1928).

⁷ Wieland, "Le Prix Nobel," Stockholm (1928).

⁸ Wieland and Vocke, *Z. physiol. Chem.*, **191**, 69 (1930).

⁹ Wieland and Deulofeu, *ibid.*, **198**, 127 (1931).

¹⁰ Borsche and Todd, *ibid.*, **197**, 173 (1931).

¹¹ Rosenheim and King, *J. Soc. Chem. Ind.*, **51**, 464 (1932).

¹² Bernal, *Nature*, **129**, 277 (1932); *J. Soc. Chem. Ind.*, **51**, 466 (1932); for summary see "Ann. Repts. Chem. Soc. (London)," Vol. 30, p. 423 (1933).

of this suggestion led both Rosenheim and King¹³ and Wieland and Dane¹⁴ to modify the perhydrochrysene to a cyclopentanoperhydrophenanthrene nucleus. This new structure for the sterols and bile acids was immediately compatible with the vast amount of experimental material which had been accumulated, and it has been tested in numerous ways since its proposal.

With the investigations of the sterols and the bile acids as a background, the structural examination of most of the other members of the group has been conducted to a satisfactory conclusion with rapidity. Although in many instances only small amounts of these natural products were available for study, degradation to mutually common compounds has been carried out in nearly all cases. As a structural study, the first interest is the nucleus. With the establishment of the nature of this ring system, organized study of its chemistry may be begun.

The Structure of the Nucleus*

As was suggested above, the structural investigation of the nucleus has been carried out exclusively with the sterols and the bile acids. The early investigators felt that the nucleus was identical in these two series, but proof was first offered by Windaus and confirmed by Wieland. With the establishment of this fact, evidence obtained from the study of cholesterol could be applied to the bile acids, and *vice versa*. Then followed a period of intensive work, in which the several rings were opened, and the products studied by thermal decomposition. This path led to a false solution, however, and it was selenium dehydrogenation which finally furnished the essential clue.

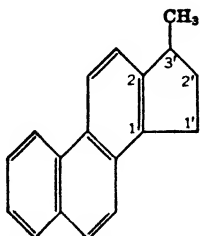
When selenium dehydrogenation of cholesterol is carried out at 360°, one of the products is a hydrocarbon, C₁₈H₁₆ (IV, Diels' hydrocarbon). Early in 1934 the structure of this hydrocarbon was definitely established as 3'-methyl-1,2-cyclopentenophenanthrene (or γ -methylcyclopentenophenanthrene), but because of the drastic conditions of selenium dehydrogenation, together with the very poor yield of product, the formation of a cyclopentenophenanthrene is not good proof of a cyclopentanoperhydrophenanthrene nucleus. Although selenium dehy-

¹³ Rosenheim and King, *Nature*, **130**, 315 (1932); *J. Soc. Chem. Ind.*, **51**, 954 (1932); **52**, 299 (1933).

¹⁴ Wieland and Dane, *Z. physiol. Chem.*, **210**, 268 (1932).

* Reviews reconciling the older work with the new structure: Windaus, *Z. physiol. Chem.*, **213**, 147 (1932); Heilbron, Simpson, and Spring, *J. Chem. Soc.*, 626 (1933); Rosenheim and King, "Ann. Rev. Biochem.," **III**, 87 (1934). Reviews giving an interesting running account of the developments are to be found in the "Ann. Repts. Chem. Soc. (London)," Vol. 24, p. 128 (1927); Vol. 25, p. 157 (1928); Vol. 28, p. 139 (1931); Vol. 30, p. 198 (1933).

drogenation of a hydrocarbon derived from certain of the bile acids to methylcholanthrene in relatively good yield furnishes confirmatory evidence of the structure of the nucleus, the real proof comes from a reinterpretation and further investigation of the oxidative degradation of cholesterol and the bile acids. Much of the evidence used in establishing the nature of the nucleus may also be employed in determining other structural details.



IV 3'-Methyl-1,2-cyclopentenophenanthrene
(Diels' hydrocarbon)

Evidence of a Common Nucleus in the Sterols and Bile Acids.

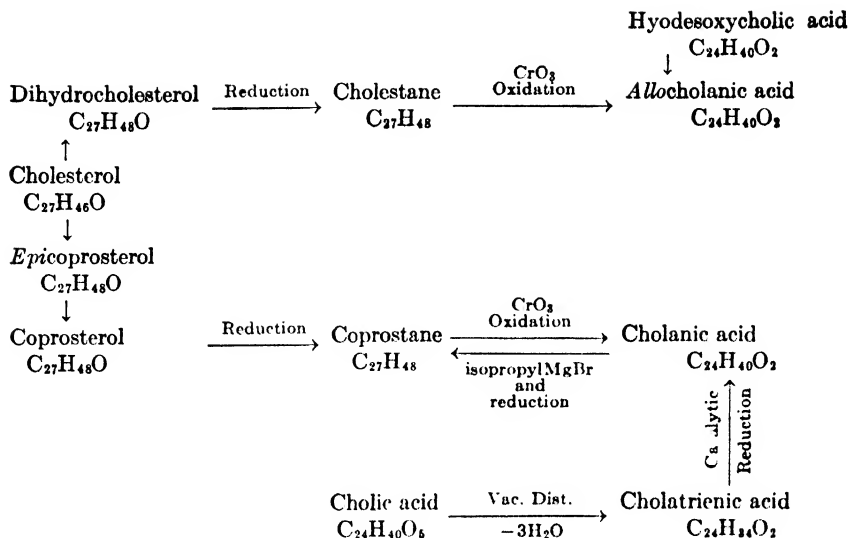
When cholesterol is catalytically hydrogenated at room temperature, dihydrocholesterol is formed; at 200°, and with nickel as a catalyst, the product is a mixture of dihydrocholesterol and two of its stereoisomers, *epidihydrocholesterol* and *epicoprosterol* (and traces of coprosterol).¹⁵ After resolving the mixture into its components, *epicoprosterol* may be converted to coprosterol. Reduction of either of these through the stage of the chloride leads to the hydrocarbon coprostane. Similarly, from dihydrocholesterol and *epidihydrocholesterol*, cholestane is obtained. Oxidation of coprostane and of its stereoisomer cholestane with hot chromic acid gives, among other products, cholic acid and *allocholic acid*, respectively.¹⁶ These two acids, the parent substances of the bile acid series, can be obtained by appropriate treatment of cholic acid or hyodesoxycholic acid. Cholic acid, for example, readily loses water when heated in high vacuum to give a triply unsaturated acid, cholatrienic acid, which by catalytic hydrogenation may be reduced to cholic acid. The conversion of hyodesoxycholic acid to *allocholic acid* is discussed under the heading Bile Acids.

Conversely, cholic acid may be converted, through the action of isopropylmagnesium bromide on its ester, to a ketone which on Clemmensen reduction gives coprostane.¹⁷ The proof of the identity of the nuclei of the two series may be summarized as shown:

¹⁵ Windaus, *Ber.*, **49**, 1724 (1916).

¹⁶ Windaus and Neukirchen, *Ber.*, **52**, 1915 (1919).

¹⁷ Wieland and Jacobi, *Ber.*, **59**, 2064 (1926).



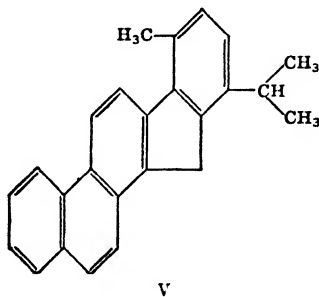
Dehydrogenation Products. The initial experiments in which cholesterol and cholic acid were dehydrogenated were conducted by Diels.¹⁸ Using palladized charcoal at *ca.* 500°, the identifiable product from cholesterol was chrysene, C₁₈H₁₂. With cholesteryl chloride at 340–360° and with selenium in place of palladized charcoal, two hydrocarbons, C₁₈H₁₆ and C₂₅H₂₄, were obtained. The latter gave reactions suggestive of a fluorene nucleus, and was thought at the time to indicate that ring IV of the old cholesterol formula (IIa) was five-membered. After Rosenheim and King had pointed out the significance of chrysene, interest in the dehydrogenation products revived, centering especially on the compound C₁₈H₁₆. Since different workers could not agree on the nature of this hydrocarbon, or even on the production of chrysene, a polemical situation developed which has led to a reasonably accurate knowledge of what happens under the drastic conditions of selenium dehydrogenation.¹⁹

Diels^{19b} and Ruzicka^{19f} in particular have examined the nature

¹⁸ Diels and Gädke, *Ber.*, **60**, 140 (1927); Diels, Gädke, and KÖrding, *Ann.*, **459**, 1 (1927); Diels and Karstens, *Ann.*, **478**, 129 (1930).

¹⁹ (a) Cook and Hewett, *J. Soc. Chem. Ind.*, **52**, 451 (1933); Cook, Hewett, Mayneord, and Roe, *J. Chem. Soc.*, 1727 (1934). (b) Diels, *Ber.*, **66**, 487, 1122 (1933); Diels and Klare, *Ber.*, **67**, 113 (1934); Diels and Stephan, *Ann.*, **527**, 279 (1937). (c) Gamble, Kon, and Saunders, *J. Chem. Soc.*, 644 (1935). (d) Raudnitz, Petrů, and Stadler, *Ber.*, **66**, 879 (1933). (e) Rosenheim and King, *J. Soc. Chem. Ind.*, **52**, 299 (1933). (f) Ruzicka, Goldberg, and Thomann, *Helv. Chim. Acta*, **16**, 812 (1933); Ruzicka, Thomann, Brandenberger, Furter, and Goldberg, *ibid.*, **17**, 200 (1934); Ruzicka and Goldberg, *ibid.*, **18**, 434 (1935). (g) Schlenk, Bergmann, and Bergmann, *J. Soc. Chem. Ind.*, **53**, 209 (1933).

of the products obtained by dehydrogenation. Cholesterol at 300–360° gives Diels' hydrocarbon and another hydrocarbon, $C_{25}H_{24}$, which, though not identical with the hydrocarbon of structure V, seems to be

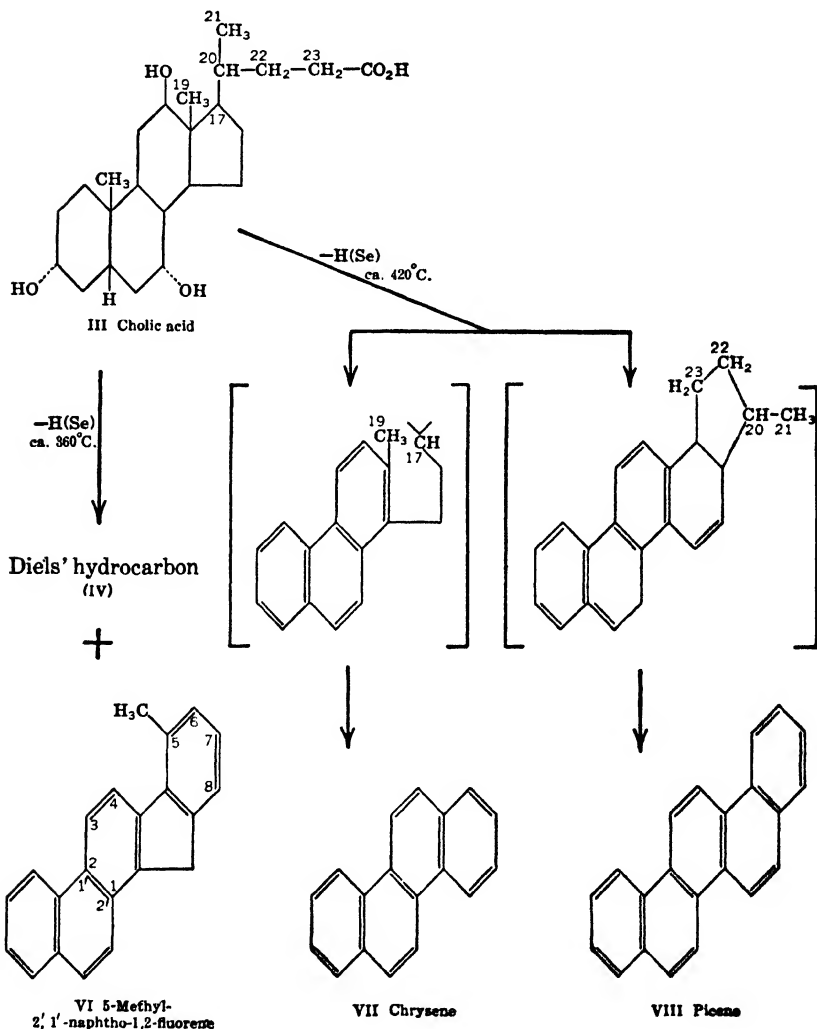


closely allied with it.^{10a} With the congeners of cholesterol, such as ergosterol and sitosterol, there is a difference of opinion about the formation of $C_{25}H_{24}$. Ruzicka finds that hydrocarbons of a greater carbon content than C_{25} are produced from these sterols, but Diels has been able to obtain only $C_{25}H_{24}$, although he admits that there may be small amounts of other hydrocarbons formed. Differences in temperature may be the explanation of the discrepancies in the results of the two workers. In the latest work of Diels the temperature of the dehydrogenations was closely regulated by carrying out the reaction in boiling acetanilide. The solution of the problem of the products of dehydrogenation is rendered difficult by the fact that the yield of each hydrocarbon is less than 1 per cent.

Cholic acid (or its dehydration product, cholatrienic acid), when subjected to selenium dehydrogenation, gives a variety of products, depending on the temperature at which the dehydrogenation is conducted. At lower temperatures (360°), Diels' hydrocarbon and a second hydrocarbon, which was first reported to be $C_{21}H_{16}$, are formed. Cook²⁰ suggested that the composition of this second hydrocarbon might be $C_{22}H_{16}$, rather than $C_{21}H_{16}$, and has proved his point by the synthesis of 5-methyl-2',1'-naphtho-1,2-fluorene (VI), which agrees well with the product obtained by Ruzicka. At temperatures of 400° or higher, chrysene (VII) and picene (VIII) are formed. Ruzicka has suggested the following mechanism for their formation: Chrysene results from the union of the angular methyl group at C_{13} with an opened ring D; picene from an analogous type of ring enlargement with simultaneous ring formation involving the side chain.

²⁰ Cook, Dansi, Hewett, Iball, Mayneord, and Roe, *J. Chem. Soc.*, 1319 (1935); Bachmann, Cook, Hewett, and Iball, *ibid.*, 54 (1936).

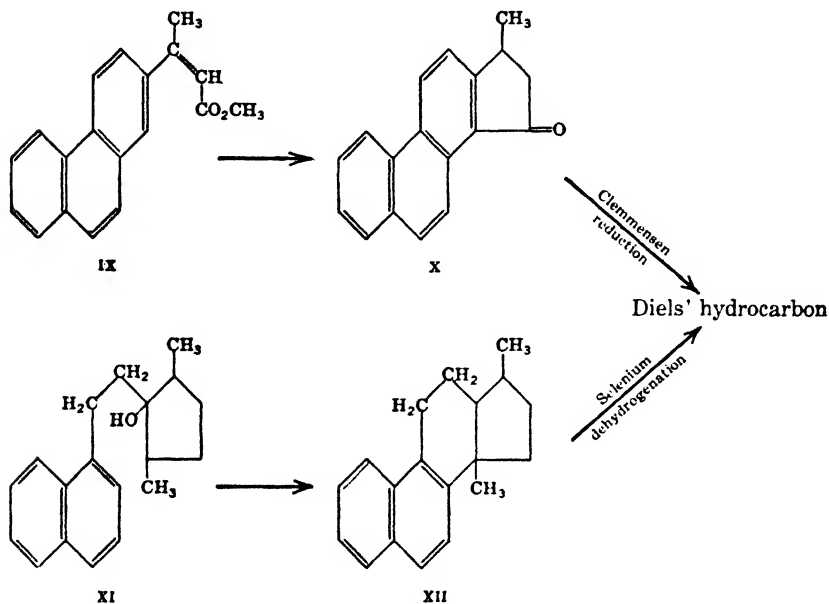
The structure of Diels' hydrocarbon as 3'-methyl-1,2-cyclopentenophenanthrene, $C_{18}H_{16}$ (IV), has been definitely established through two syntheses. By the first of these,²¹ 2-acetylphenanthrene is con-



densed with bromoacetic ester, the product (IX) hydrolyzed, reduced, and converted through the acid chloride to the cyclic ketone (X). Clemmensen reduction of the ketone gives Diels' hydrocarbon. In the

²¹ Bergmann and Hillemann, *Ber.*, **66**, 1302 (1933); Hillemann, *Ber.*, **68**, 102 (1935); **69**, 2610 (1936); cf. Diels and Rickert, *Ber.*, **68**, 325 (1935).

second synthesis,²² β -(1-naphthyl)-ethylmagnesium bromide is reacted with 2,5-dimethylcyclopentanone to give an alcohol (XI) which after dehydration with phosphorus pentoxide is cyclized to yield the hydrocarbon, XII. Selenium dehydrogenation of XII gives 3'-methyl-1,2-cyclopentenophenanthrene. Identification of the end product with the



sterol hydrocarbon had to be made by an elaborate series of physical measurements, as well as by means of the picrate and other addition products, because the hydrocarbon does not give melting-point depression when mixed with structurally similar compounds. Diels' hydrocarbon as obtained from sterols by dehydrogenation has a magnificent blue fluorescence, which is absent in the synthetic product. Both preparations react with bromine to give a well-defined tribromide,²³ and with nitrous acid to form an isonitroso compound of uncertain structure. The characterization of this hydrocarbon is of great importance, for its formation by selenium dehydrogenation serves as one of the most convenient ways of discovering new members of the cyclopentanoperhydrophenanthrene group.

The ring enlargement that takes place with selenium dehydrogenation at temperatures above 400° has attracted some interest. Model

²² Harper, Kon, and Ruzicka, *J. Chem. Soc.*, 124 (1934).

²³ Diels and Rickert, *Ber.*, **68**, 267 (1935).

experiments on the α - and β -methyl- and ethyl-hydrindenes show that they undergo ring enlargement to produce naphthalene or methyl-naphthalene at temperatures of 450°, but not at lower temperatures.²⁴ The absence of ring enlargement at lower temperatures has been confirmed by other model experiments on several related hydrindenes.²⁵ Diels' hydrocarbon, however, does not give chrysene when treated with selenium or palladium at 450°.

Methylcholanthrene. The formation of Diels' hydrocarbon from the sterols and the bile acids suggests the nature of the nucleus, but the extremely poor yields obtained by dehydrogenation weaken the proof that the nucleus is cyclopentanoperhydrophenanthrene. Two of the bile acids, however, can be converted to methylcholanthrene in relatively good yield. Since the structure of methylcholanthrene can be established by degradation and by synthesis, this transformation materially strengthens the proof of the nature of the nucleus.

From cholic or desoxycholic acid, 12-ketocholanic acid (XIII) is obtained by methods which will be discussed later (p. 1248). Pyrolysis of the ketocholanic acid gives the hydrocarbon dehydronorcholene (XIV), and selenium dehydrogenation of the latter to methylcholanthrene proceeds in a yield of 30 per cent.²⁶ On oxidative degradation methylcholanthrene is converted to 5,6-dimethyl-1,2-benzanthraquinone (XVI), which, in turn, is characterized by further oxidation to 1,2,5,6-anthraquinonetetracarboxylic acid.²⁷ In the synthesis²⁸ of methylcholanthrene, a five-membered ring is formed on *p*-bromotoluene, giving a bromomethylhydrindene. The Grignard (p. 417) compound (XVIII) from this hydrindene is then reacted with α -naphthoyl chloride (XVII) to give a ketone (XIX) which on pyrolysis yields methylcholanthrene. By this transformation and synthesis, not only is the presence of a five-membered ring in appropriate sequence to three six-membered rings shown, but the attachment of the principal side chain of the bile acids at C₁₇ is also established.

Relationship of the Hydroxyl Group and Double Bond in Cholesterol. The hydroxyl group and the double bond in cholesterol (II) are the chief points of attack in its degradation. By examination of the oxidation products these two functions have been found to be present in two

²⁴ Ruzicka and Peyer, *Helv. Chim. Acta*, **18**, 676 (1935).

²⁵ Clemo and Dickenson, *J. Chem. Soc.*, 735 (1935); Chuang, Ma, and Tien, *Ber.*, **68**, 1946 (1935).

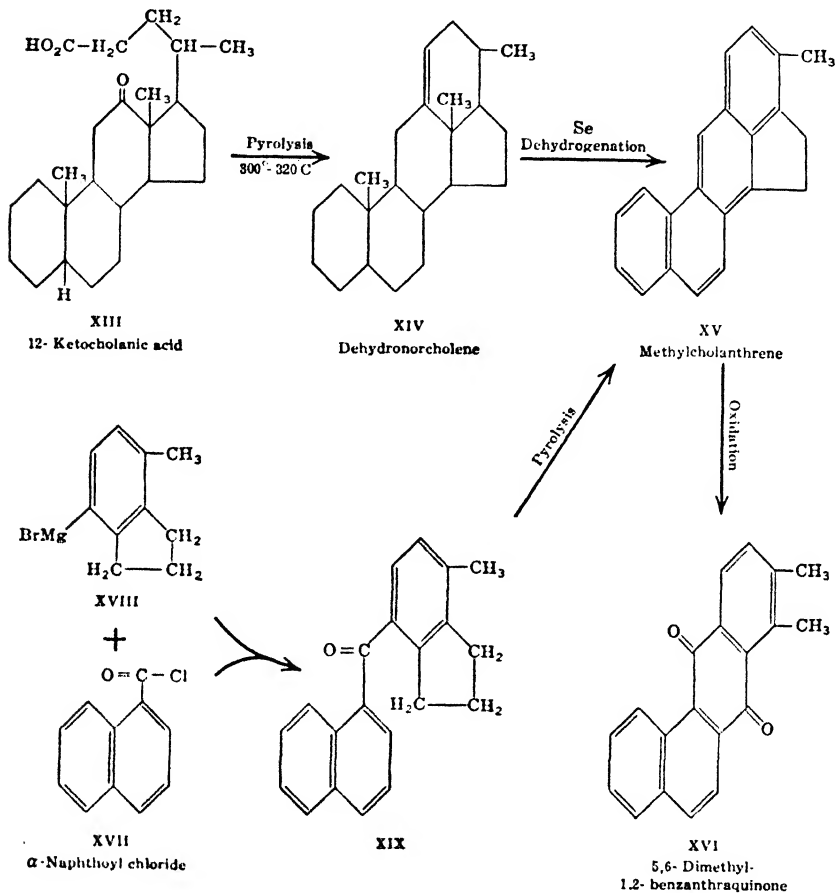
²⁶ Cook and Haslewood, *J. Chem. Soc.*, 428 (1934).

²⁷ Wieland and Wiedersheim, *Z. physiol. Chem.*, **186**, 229 (1930); Wieland and Dane, *ibid.*, **219**, 240 (1933); Cook and Haslewood, *J. Soc. Chem. Ind.*, **52**, 758 (1933); Cook, Hewett, and Haslewood, *ibid.*, **52**, 949 (1933).

²⁸ Fieser and Seligman, *J. Am. Chem. Soc.*, **57**, 228, 942 (1935).

different rings in an α,γ -system, the hydroxyl group being located at C_3 and the double bond at $C_5 : C_6$. The evidence follows:

1. Nitration of cholesteryl acetate yields a nitrocholesteryl acetate in which the nitro group is presumably attached at C_6 .²⁹ Reduction

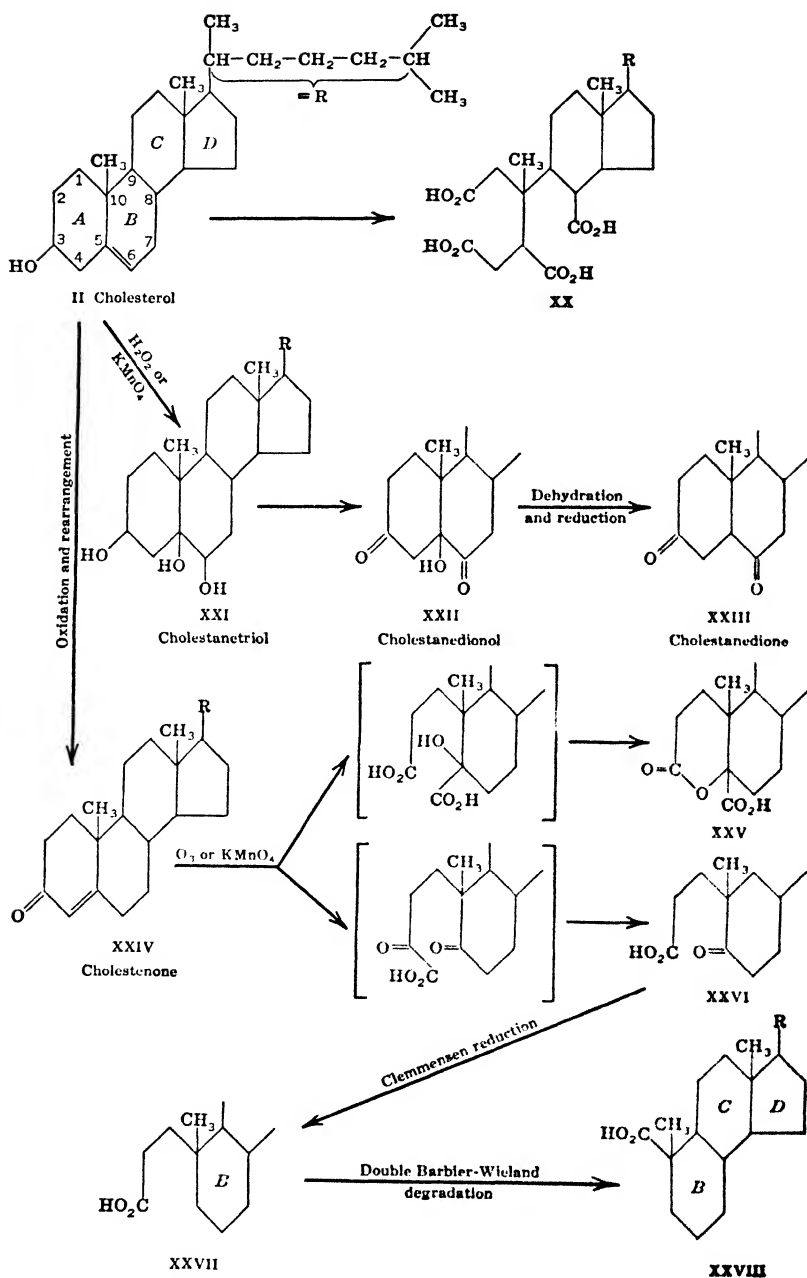


of the nitro compound with zinc and acetic acid yields, with elimination of the nitro group, cholestanonol.³⁰ Oxidation of the latter gives rise through the stages of cholestanedione (XXIII) and a ketodicarboxylic acid to the tetracarboxylic acid XX.³¹ In this degradation, ring A is opened first and then ring B. By a somewhat different procedure—conversion of cholestanonol to chlorcholestanone—ring B may be

²⁹ Mauthner and Suida, *Monatsh.*, **15**, 85 (1894); **24**, 648 (1903).

³⁰ Windaus, *Ber.*, **36**, 3752 (1903).

³¹ Windaus and v. Staden, *Ber.*, **54**, 1059 (1921).



opened first and then ring A.³² With either procedure the same tetracarboxylic acid (XX) results. Assuming that no rearrangements occur, the above transformations indicate that the hydroxyl group and the double bond are contained in separate rings.

2. Oxidation of cholesterol by potassium permanganate, hydrogen peroxide, or perbenzoic acid yields two isomeric cholestanetriols, $C_{27}H_{48}O_3$ (XXI), which on further oxidation give two isomeric hydroxy diketones, $C_{27}H_{44}O_3$ (XXII).³³ Dehydration and reduction convert both these ketones to the same cholestanedione (XXIII). The properties of this dione are those of a γ -diketone. It reacts with hydrazine to form a pyridazine;³⁴ the ketodicarboxylic acid formed from the diketone by oxidation is very stable and definitely not a β -keto acid, since the corresponding hydroxy acid obtained by reduction of the carbonyl group readily lactonizes.³⁵ These reactions show that the hydroxyl and double bond form an α,γ -system.

3. On heating cholesterol to 290° with copper oxide, cholestenone (XXIV) is formed.³⁶ Better yields are obtained, however, by cold two-phase oxidation of cholesterol dibromide with permanganate or chromic acid, followed by debromination with zinc³⁷ or sodium iodide.^{38*} Cholestenone has an absorption spectrum (maximum $240\text{ m}\mu$) which indicates that the carbonyl and double bond form a conjugated system.³⁹ When cholestenone is oxidized with ozone or potassium permanganate, two products result: an acid of composition $C_{27}H_{44}O_4$ (XXV), and, as the principal product, a keto acid, $C_{26}H_{44}O_3$ (XXVI), formed with the loss of carbon dioxide.⁴⁰ The production of these two acids is satisfactorily explained only if a structure with the carbonyl and the double

³² Windaus and Stein, *Ber.*, **37**, 3699 (1904).

³³ Windaus, *Ber.*, **40**, 257 (1907); Pickard and Yates, *J. Chem. Soc.*, **93**, 1678 (1908); Westphalen, *Ber.*, **48**, 1064 (1915); Criegee, *Ber.*, **65**, 1770 (1932).

³⁴ Windaus, *Ber.*, **39**, 2249 (1906).

³⁵ Windaus and Hossfeld, *Z. physiol. Chem.*, **145**, 177 (1925).

³⁶ Diels and Abderhalden, *Ber.*, **37**, 3092 (1904); Windaus, *Ber.*, **39**, 518 (1906).

³⁷ Windaus, *Ber.*, **39**, 518 (1906); Ruzicka, Brüngger, Eichenberger, and Meyer, *Helv. Chim. Acta*, **17**, 1407 (1934).

³⁸ Schoenheimer, *J. Biol. Chem.*, **110**, 461 (1935).

* Even more satisfactory than these methods is one that has been developed by Oppenauer, *Rec. trav. chim.*, **56**, 137 (1937). Cholesterol (or any steroid alcohol) when heated with a large excess of a ketone like acetone in the presence of an aluminum tertiary alkoxide is dehydrogenated to the corresponding ketone or its rearrangement product. If the reaction is carried out in benzene, yields of 80-90 per cent are easily obtained, but in lower-boiling solvents the results are not as favorable. The method is essentially the reverse of the Meerwein [Ann., **444**, 221 (1925)]-Ponndorf [Angew. Chem., **39**, 138 (1926)] method of reducing carbonyl groups to carbinols.

³⁹ Menschick, Page, and Bossert, *Ann.*, **495**, 225 (1932); Mohler, *Helv. Chim. Acta*, **20**, 289 (1937).

⁴⁰ Lettré, *Z. physiol. Chem.*, **221**, 73 (1933).

4. Reduction of the carbonyl group of the keto acid, $C_{26}H_{44}O_3$ (XXVI), by the Clemmensen method gives the acid $C_{26}H_{46}O_2$ (XXVII). This acid may be degraded stepwise by a method (Barbier-Wieland degradation) that in effect counts the methylene groups following a carboxyl group.* The steps involved are:

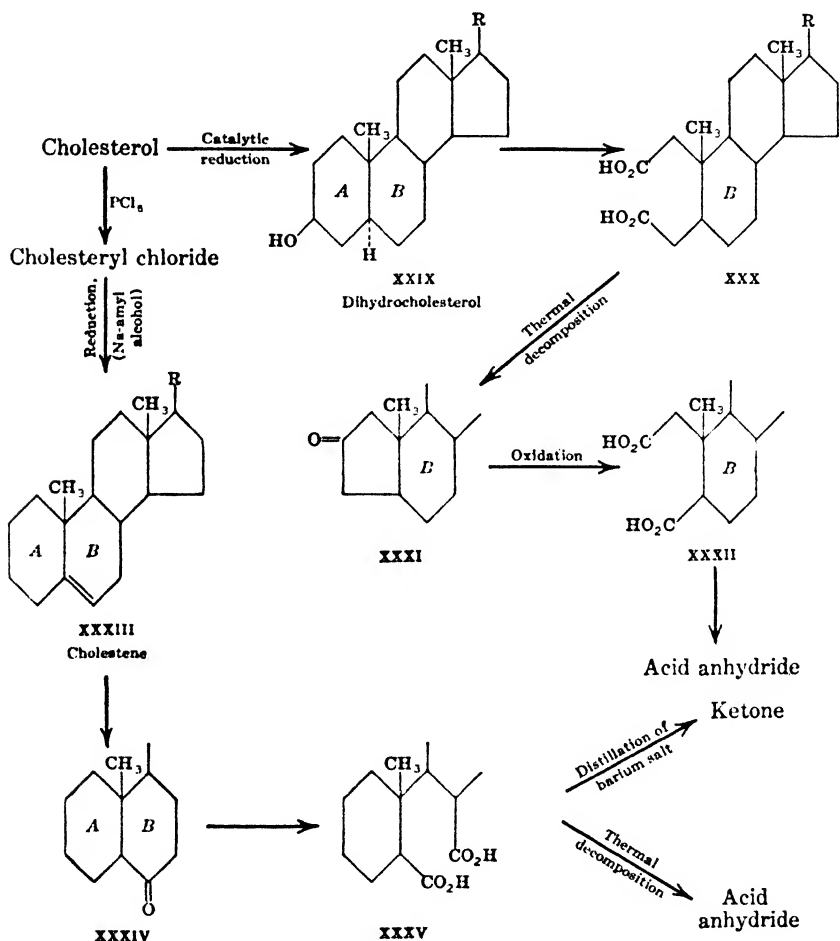
By this means the acid XXVII after two degradations yields the acid $C_{24}H_{42}O_2$ (XXVIII), which cannot be degraded further;⁴² thus, two methylene groups are diagnosed. The product (XXVIII) yields carbon monoxide easily when heated with concentrated sulfuric acid, and forms esters with difficulty; this indicates that the carboxyl group is attached to a quaternary carbon. Since the carboxyl group of the keto acid (XXVI) originated from the carbonyl of cholestenone, the position of the hydroxyl group of cholesterol is placed as being three carbons removed from this quaternary carbon, i.e., at C_3 .

The Size of Rings A and B. The size of rings A and B has been determined by examination of the dicarboxylic acids produced by opening each ring separately. According to Blanc's rule,⁴³ when dicarboxylic acids are heated with acetic anhydride and distilled, or simply distilled (thermal decomposition), ketones result from 1,6- or 1,7-dicarboxylic acids while 1,4- and 1,5-diacids produce anhydrides.

* This method of degradation was originally developed by Barbier and Loquin [*Compt. rend.*, **156**, 1443 (1913)] as a general process for degrading acids. It was later employed by Wieland, e.g. Wieland, Schlichting, and Jacobi, reference 49, but apparently without knowledge of Barbier's earlier work. Although the method is usually referred to as the Wieland degradation, it seems more appropriate to designate it as the Barbier-Wieland degradation.

⁴² Blanc, *Bull. soc. chim.*, [3] **33**, 893 (1905).

Dihydrocholesterol (XXIX), the product of catalytic hydrogenation of cholesterol at room temperature, is readily oxidized to a dicarboxylic acid, $C_{27}H_{46}O_4$ (XXX). When subjected to thermal decomposition, the latter yields, with loss of water and carbon dioxide, a cyclic ketone, $C_{26}H_{44}O$ (XXXI). This ketone on oxidation gives rise to another



dicarboxylic acid, $C_{26}H_{44}O_4$ (XXXII), but from this diacid the acetic anhydride treatment produces an acid anhydride and not a ketone.⁴⁴ Ring A, which is opened in the oxidation, is clearly six-membered.

When the ring containing the double bond is opened in a similar

⁴⁴ Windaus and Dalmer, *Ber.*, **52**, 162 (1919); Windaus, Rosenbach, and Riemann, *Z. physiol. Chem.*, **130**, 113 (1923).

manner, somewhat different results are obtained.⁴⁵ Cholesterol is converted to cholestene (XXXIII) by reducing cholesteryl chloride with sodium and amyl alcohol. Nitration and reduction form a ketone, heterocholestanone (XXXIV), which, on oxidation, is converted into a dicarboxylic acid, $C_{27}H_{46}O_4$ (XXXV). But when this dicarboxylic acid is subjected to thermal decomposition, it forms an anhydride and not a ketone. The formation of the anhydride from the diacid was interpreted for many years as proof that ring B was a five-membered ring (structure IIa, ring II). Reëxamination of the situation has disclosed a number of loopholes. In the first place, it has been shown that substituted adipic acids do not always behave as might be expected;⁴⁶ and, secondly, Stange⁴⁷ has found that the barium salt of the diacid (XXXV) does form a ketone. The discrepancy between fact and theory in this case has led Wieland and Dane¹⁴ to modify Blanc's rule (pp. 16, 1240) to apply only to those compounds in which the carboxyl groups are attached to the same ring; for example, the dicarboxylic acids formed by opening rings B and C (see structure I) would be expected to behave anomalously.*

From another series of reactions supplementary evidence as to the nature of ring B may be obtained.⁴⁸ Oxidation of cholesterol with hypobromite converts it to an unsaturated dicarboxylic acid, $C_{27}H_{44}O_4$ (XXXVI, Diels' acid), which may be progressively oxidized through a keto diacid (XXXVII), a diketo triacid (XXXVIII), to a tricarboxylic acid, $C_{25}H_{42}O_6$ (XXXIX). When subjected to thermal decomposition the tricarboxylic acid loses carbon dioxide and water and forms a keto-monocarboxylic acid, $C_{24}H_{40}O_3$ (XL). Opening of the newly formed ring by oxidation gives a tricarboxylic acid, $C_{24}H_{40}O_6$ (XLI). This acid likewise forms a keto acid (XLII) when treated with acetic anhydride, thus demonstrating a 1,6-dicarboxylic acid. Since in the transformation to the tricarboxylic acid, XLI, three carbon atoms are lost as carbon dioxide, and the end product can be converted to a keto acid, both rings A and B must have been six-membered.

The Size of Ring D. Dehydration of any of the bile acids is readily effected by distillation in high vacuum. The resulting unsaturated

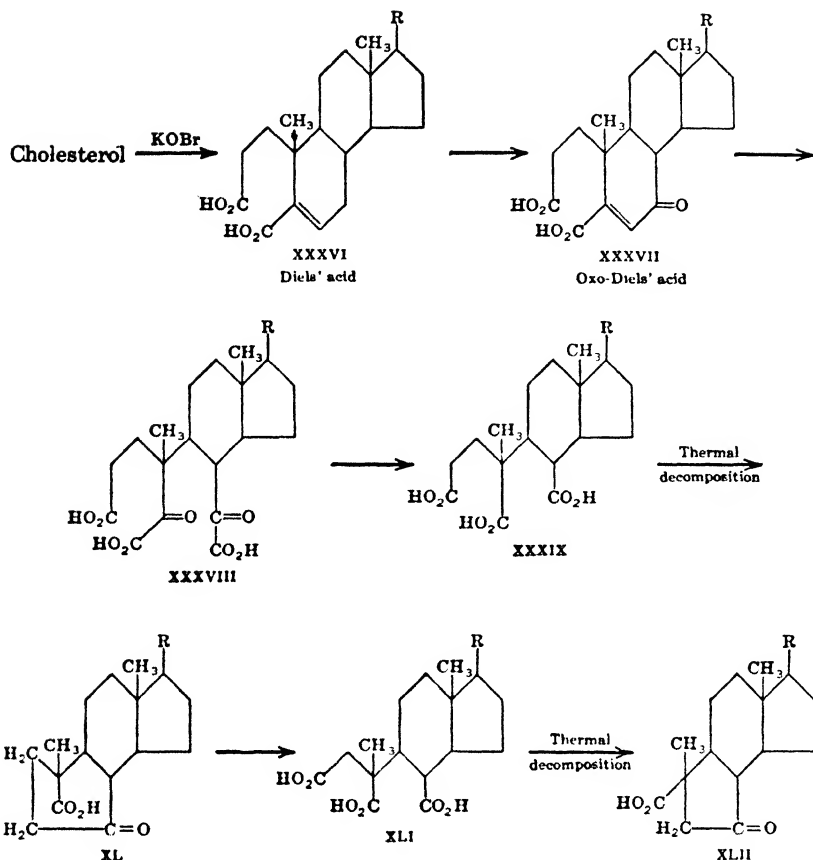
⁴⁵ Windaus and Dalmer, reference 44; Windaus, *Ber.*, **53**, 488 (1920).

⁴⁶ Farmer and Kracovski, *J. Chem. Soc.*, 680 (1927). Cf. Hill, *J. Am. Chem. Soc.*, **52**, 4110 (1930).

⁴⁷ Stange, *Z. physiol. Chem.*, **218**, 74 (1933).

* A model research by Vocke [*Ann.*, **508**, 1 (1934)], corroborated by Hückel [*Ann.*, **508**, 10 (1934)] as a note at end of Vocke's article just cited, carried out on hydrodiphenic acid does not bear out the modified rule, for in this case satisfactory ketone formation is observed. The spatial configuration of the carboxyl groups is perhaps the deciding factor [Rusicka, Furter, and Thomann, *Helv. Chim. Acta*, **16**, 327 (1933)].

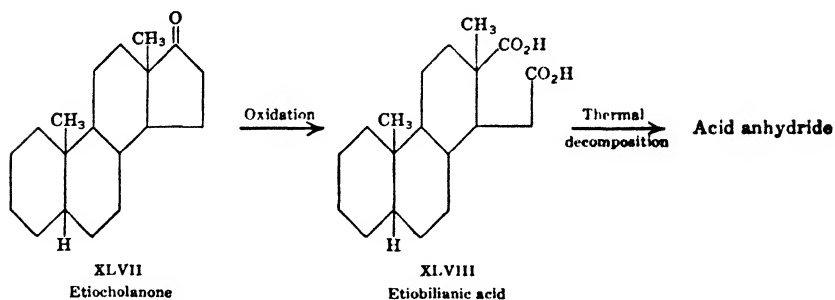
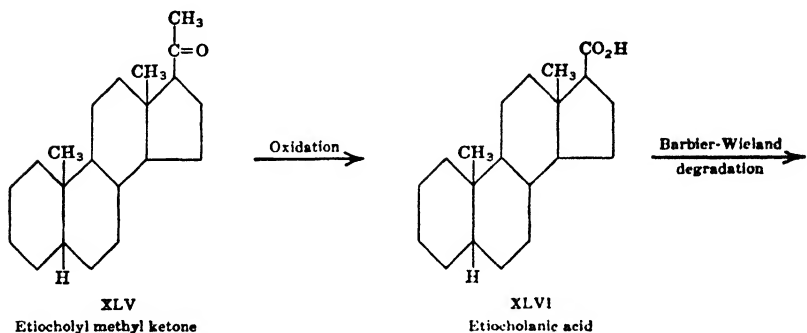
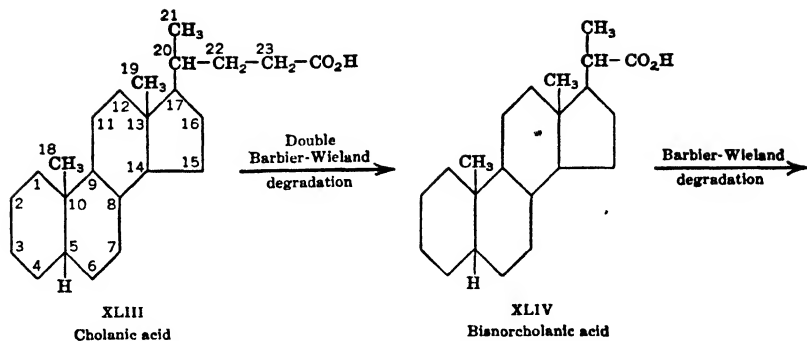
⁴⁸ Diels and Abderhalden, *Ber.*, **36**, 3179 (1903); Windaus, *Ber.*, **41**, 611, 2558 (1908); **42**, 3770 (1909); **45**, 1316, 2421 (1912).



acids may be catalytically hydrogenated to the parent cholanolic acid (XLIII). By means of the Barbier-Wieland degradation and oxidation, cholanolic acid can be degraded stepwise⁴⁹ through the following stages: cholanolic acid (C₂₄) → norcholanolic acid (C₂₃) → bisnorcholanolic acid (C₂₂, XLIV) → etiocholyl methyl ketone* (C₂₁, XLV) → etiocholanolic acid (C₂₀, XLVI) → etiocholanone (C₁₉, XLVII) → etiobilanic acid (C₁₉, XLVIII, a dicarboxylic acid). These reactions demonstrate the

⁴⁹ Wieland, Schlichting, and Jacobi, *Z. physiol. Chem.*, **161**, 80 (1926).

* Etiocholyl methyl ketone was not obtained by Wieland by degradation but as a by-product from the oxidation mixtures of the previous steps. Bisnorcholanolic acids have been degraded to etiocholyl methyl ketones by a number of other workers, however. For recent publications see Shimizu and Kasuno, *Z. physiol. Chem.*, **244**, 167 (1936), and Morsman, Steiger, and Reichstein, *Helv. Chim. Acta*, **20**, 1 (1937). The data of Reichstein show that 100 g. of cholic acid yields about 40 g. of norcholic acid and, by succeeding degradation, 10 g. of bisnorcholic acid and 1.5 g. of trihydroxyetiocholyl methyl ketone.



CH_3
 $|$

presence of a side chain, $-\text{CH}-\text{CH}_2-\text{CH}_2-\text{CO}_2\text{H}$, attached to a ring. The transformation of etiocholanone to etiocholanone without loss of carbon shows that this attachment is through a tertiary carbon, and that adjacent to the tertiary carbon there is a methylene group. Thermal decomposition of the end product of oxidation, etiocholanone, gives an acid anhydride and not a ketone. Since the ring is opened without

loss of carbon, and with the production of an acid that behaves like glutaric acid, a five-membered ring is indicated. Because of the failure of Blanc's rule, the formation of an anhydride is not adequate proof that ring D is five-membered. But when the evidence from the dehydrogenation experiments, particularly the formation of methylcholanthrene, is added to this degradation, the proof is convincing.

The Degradation of Lithocholic Acid. When lithocholic acid (XLIX), 3-hydroxycholan-2-one, is oxidized with nitric acid, the ring bearing the hydroxyl is opened with the production of two isomeric tricarboxylic acids, lithobilianic (L) and isolithobilianic acid (LI), formed by the rupture of bonds on different sides of the hydroxyl group.⁵⁰ These two acids are identical with a pair formed by stepwise oxidation of coprosterol. In the first step ring A is opened with the formation of two dicarboxylic acids, one of which is less soluble than the other. Further oxidation removes the isopropyl group of the isoöctyl side chain, with the production of lithobilianic acid from the less soluble dicarboxylic acid and isolithobilianic acid from the other acid.⁵¹ As would be expected, thermal and oxidative degradation proceed through stages analogous to those described for cholesterol (XXXVI-XLII). The transformation is summarized in structures LII-LV. The end product, the tetracarboxylic acid, $C_{21}H_{32}O_8$ (LV), is identical with an acid obtained by Windaus⁵² from the oxidation of the tricarboxylic acid XLI formed in the degradation of cholesterol. Thermal decomposition of LV produces a pyroketodicarboxylic acid (LVI), thus showing the presence of an adipic acid system. This keto acid, when oxidized, passes through the stage of a malonic acid to a tricarboxylic acid (LVII) from which only an anhydride can be formed. The end product must contain a glutaric acid system, while the malonic acid from which it was formed demonstrates a branching of the chain. On inspection it is apparent that the branching takes place at the quaternary carbon atom diagnosed in the degradation of cholestenone. Production of the ketodicarboxylic acid (LVI) constitutes further proof that ring B is six-membered, for otherwise anhydride formation would occur.

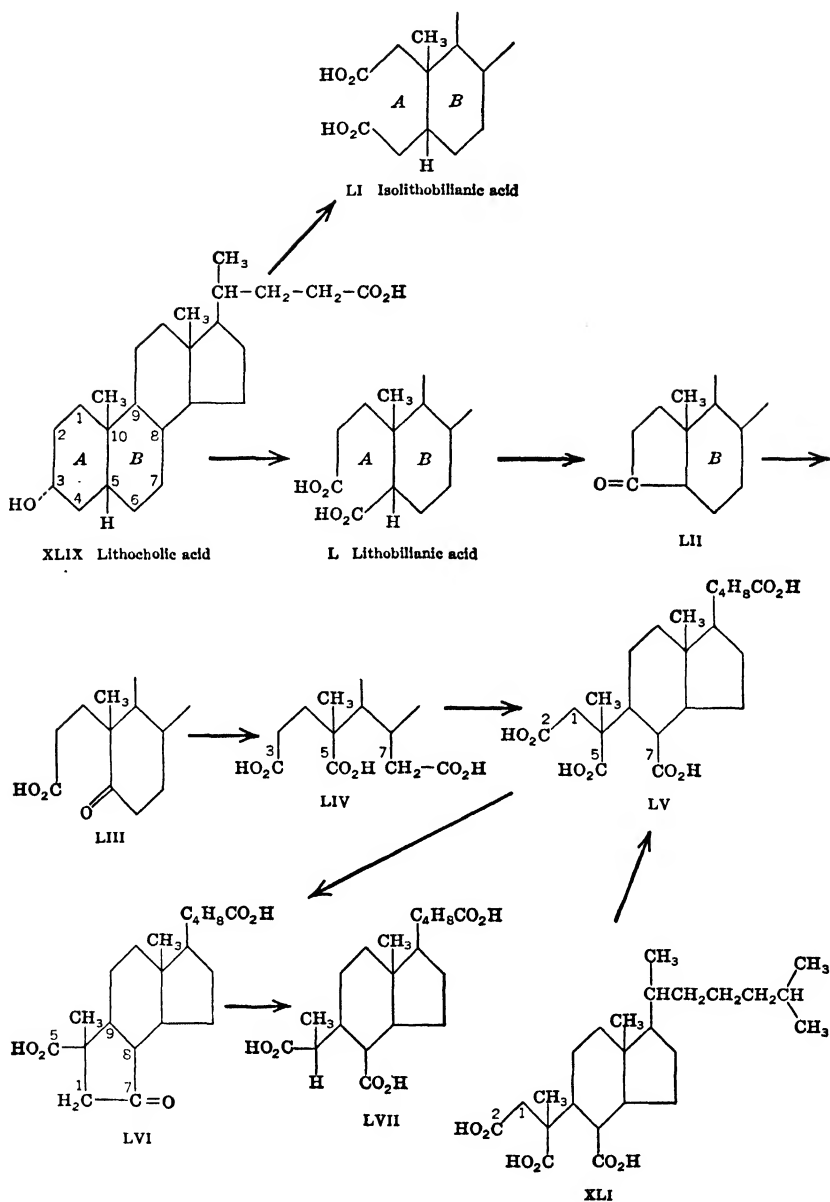
The Degradation of Desoxycholic Acid. Chromic acid oxidation in the cold of desoxycholic acid (LVIII) converts it to the corresponding diketone acid, dehydrodesoxycholic acid (LIX); further oxidation by means of nitric acid opens up one ring to form desoxybilianic acid, a ketotricarboxylic acid (LX), which by Wolff-Kishner * reduction is con-

⁵⁰ Wieland and Weyland, *Z. physiol. Chem.*, **110**, 123 (1920).

⁵¹ Langer, *ibid.*, **216**, 189 (1933). Cf. Windaus and Riemann, *ibid.*, **126**, 277 (1923).

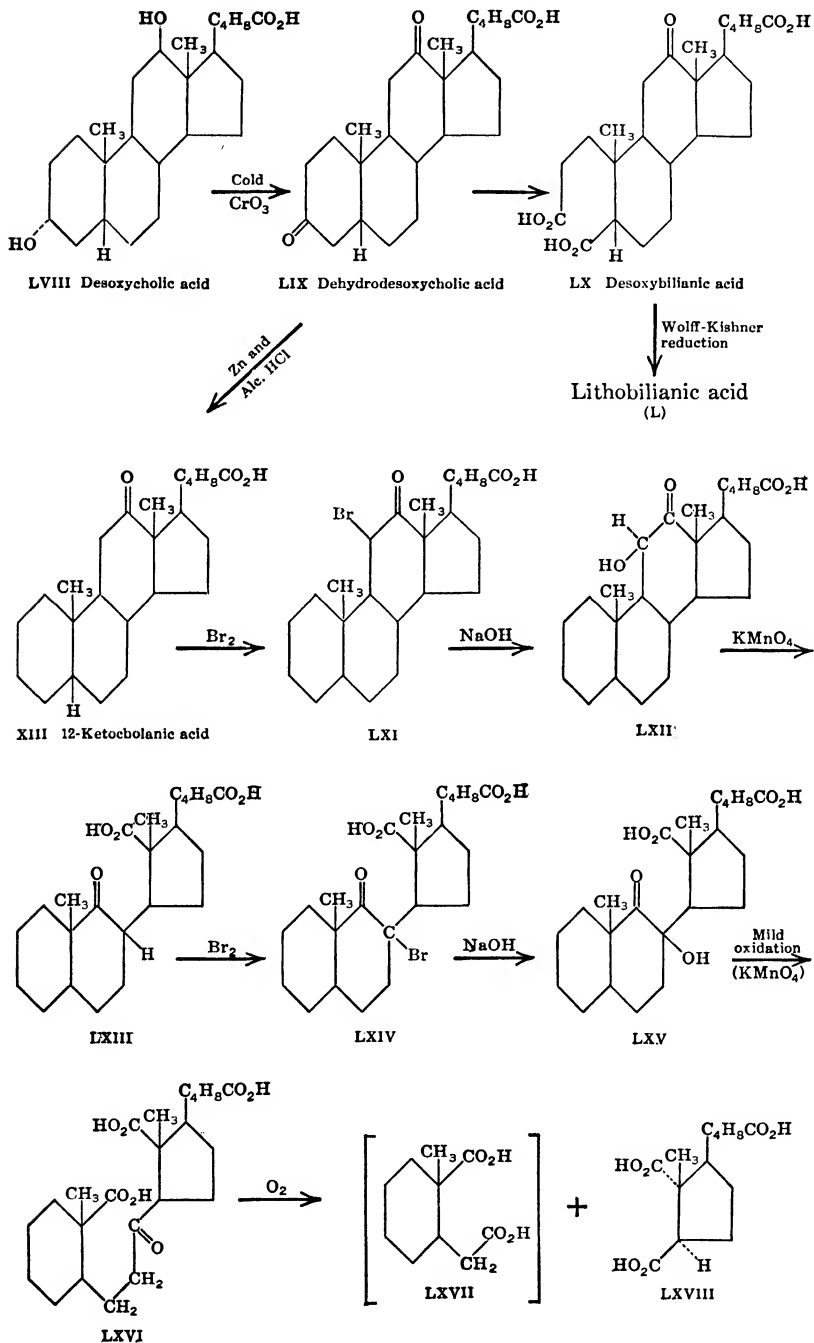
⁵² Wieland, Dane, and Scholz, *ibid.*, **211**, 261 (1932).

* Wolff-Kishner reduction: The reduction of a carbonyl group to methylene by heating the hydrazone or semicarbazone with sodium ethylate in ethyl alcohol at ca. 180° (p. 554).



verted to lithobillanic acid (L).⁵³ One of the hydroxyl groups of desoxycholic acid must, therefore, be attached at C₃ as in lithocholic acid and cholesterol. If dehydrodesoxycholic acid (LIX) is treated with

⁵³ Wieland and Kuhlenkampff, *ibid.*, **108**, 295 (1920).



zinc and alcoholic hydrochloric acid, the carbonyl at C₃ is reduced to methylene, with the formation of 12-ketocholanic acid (XIII) which is resistant to nitric acid oxidation. By bromination and subsequent hydrolysis, an hydroxyl group may be introduced on a carbon adjacent to the carbonyl. The resulting hydroxy ketone is readily oxidized with loss of carbon to a ketodicarboxylic acid (LXIII). Repetition of this process gives rise to a ketotricarboxylic acid (LXVI) and finally to the acid C₁₃H₂₀O₆ (LXVIII).⁵³ The other fragment (LXVII) has not been isolated. The structure of the important triacid, C₁₃H₂₀O₆ (LXVIII), follows from another oxidative procedure.

The Structure of Acid C₁₃H₂₀O₆. When desoxycholic acid is acted upon by a mixture of concentrated nitric and sulfuric acids (mixed acids) in the cold, it passes through the stage of a diketodicarboxylic acid (LXIX) to give a tetrabasic acid, C₁₆H₂₄O₈ (LXXI), and 1,3,3-butanetricarboxylic acid (LXX) as a by-product. Thermal decomposition of the tetrabasic acid (LXXI) gives in low yield a pyroketodicarboxylic acid (LXXII) which on oxidation is converted through a malonic acid (LXXIII) to the acid C₁₃H₂₀O₆.⁵⁴ Clemmensen reduction (p. 553) of the pyro ketone (LXXII) transforms it into a dicarboxylic acid. The diester of this dicarboxylic acid reacts with phenylmagnesium bromide in such a way that but one ester group is converted to a carbinol. Barbier-Wieland degradation of the carbinol shows that the reactive carbethoxy group is present in the side chain

CH₃
|
—CH—CH₂—CH₂—CO₂H, found in desoxycholic acid.⁸

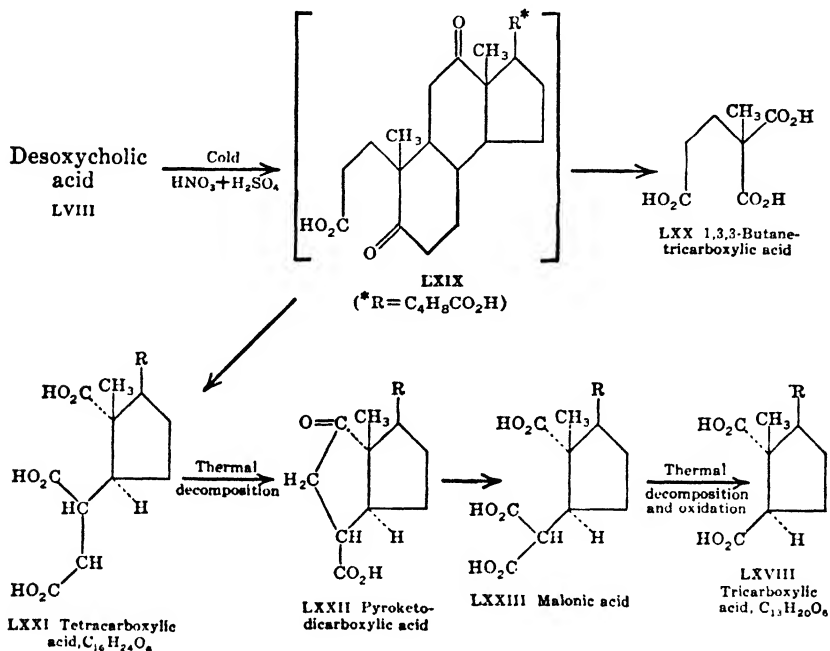
This side chain must be present in the acid C₁₃H₂₀O₆, also. Since the side chain is known to be attached to a five-membered ring, and three carboxyl groups may be detected by titration, only the fragment —CH₃ remains to be placed. That this fragment is a methyl group attached at C₁₃ follows from an ingenious argument of Wieland and Dane.⁵⁵

The acid C₁₃H₂₀O₆ (LXVIII) readily forms an anhydride; saponification of the anhydride does not return the original acid, but an isomer which has a lower melting point and greater solubility. Evidently a rearrangement from a *trans* to a *cis* form occurs. But if LXVIII has a *trans* structure, then the acid C₁₆H₂₄O₈ (LXXI) must have a *trans* structure. The low yield of pyro ketone (LXXII) is thus accounted for, and an interesting question of isomerism is raised, for the ketone is evidently a decalin-like compound containing two cyclopentane rings in

⁵⁴ Wieland and Schlichting, *ibid.*, **134**, 276 (1924); Wieland and Voecke, *ibid.*, **177**, 68 (1929).

⁵⁵ Wieland and Dane, *ibid.*, **216**, 91 (1933).

the *trans* position. Such a system has not been investigated, but according to Hückel⁵⁶ the *cis* form should be strain-free, while the *trans* modification should exhibit a moderate degree of strain (p. 50). The investigations of Windaus⁵⁷ have shown that when a five-membered ring



is formed on a cyclohexane ring by thermal decomposition of dicarboxylic acids a *cis* form results, and in no case where attachment is through a secondary ring carbon is the *trans* form produced. But in the formation of the pyroketo, the *trans* configuration persists unchanged, rearrangement being prevented through the influence of some other group. A methyl group attached at C_{13} would exert such an influence, while if it were attached at C_{14} it would not prevent rearrangement.

The production of the acid $\text{C}_{13}\text{H}_{20}\text{O}_6$ constitutes a proof that the second hydroxyl of desoxycholic acid is attached at C_{12} . Since the demonstrable five-membered ring comes through the oxidation unscathed, the second hydroxyl could not have been attached to it. Examination of the other dihydroxycholanolic acids excludes the possibility of attachment to ring B. In ring C only two positions, C_{11} and C_{12} , can

⁵⁶ Hückel, "Theoretische Grundlagen der organischen Chemie," Akademische Verlagsgesellschaft, Leipzig (1931), Vol. I, p. 63.

⁵⁷ Windaus, Hückel, and Reverey, *Ber.*, **56**, 91 (1923); Windaus, *Ann.*, **447**, 233 (1926).

be considered, and only the latter is compatible with the behavior on bromination. For example, if XIII were 11-ketocholanic acid, the result of the first bromination (LXI-LXII) would be a tricarboxylic acid.

The other method (LXIX-LXXIII) of producing the acid $C_{13}H_{20}O_8$, though not affording as good direct evidence, serves as supplementary proof. If LXIX were an 11-keto compound, a $-CH_2-CO_2H$ group would be formed on C_{13} as one of several products of the oxidation. Actually the oxidation proceeds in relatively good yield to form acid $C_{16}H_{24}O_8$, and the behavior of this acid is such that other structures cannot be considered; the formation of the malonic acid LXXIII, for example, confirms the branching of the chain at C_8 .

The Side Chains. The point of attachment, C_{17} , of the principal side chain to the nucleus was suggested from x-ray and surface film measurements (pp. 1229, 1758),¹² and confirmed by the formation of methyl-cholanthrene from 12-ketocholanic acid.²⁶ The evidence on this point has been discussed and may be regarded as satisfactory.

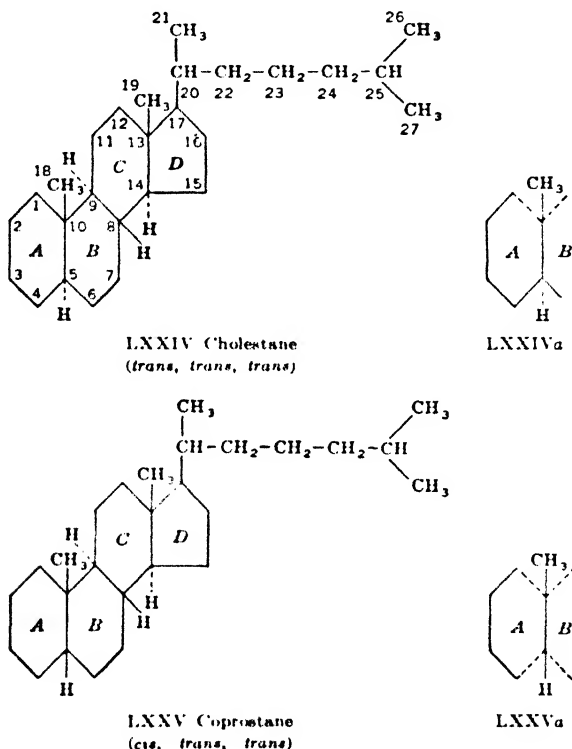
After providing for the carbon and hydrogen requirements of the nucleus and the side chain of the sterols and bile acids, there remain two carbons and six hydrogens to be attached. These have been placed as methyl groups at C_{10} and C_{13} . The evidence indicating attachment of one of these at C_{13} is given above. For some time certain of the English school of investigators favored attachment at C_{14} rather than at C_{13} , since such a structure fitted in with a postulated biological formation of cholesterol from isoprene units (p. 8).⁵⁸ The argument collapsed, however, when applied to ergosterol and stigmasterol, which are alkylated at C_{24} , and the position at C_{14} is no longer seriously considered.

Proof that the second methyl group is attached at C_{10} is much more direct. The by-product of the nitric acid oxidation of desoxycholic acid, the tricarboxylic acid $C_7H_{10}O_6$ (LXX), loses carbon dioxide when heated and forms α -methylglutaric acid. With this as a clue, synthesis has established the structure of LXX as 1,3,3-butanetricarboxylic acid.⁸ The formation of this acid shows the presence of a quaternary carbon atom bearing a methyl group. Since C_{10} is known to be quaternary from the degradation of cholestenone, the proof of the attachment of a methyl group at this point seems to be conclusive, for the tricarboxylic acid must result from rings A and B in the oxidation of desoxycholic acid.

⁵⁸ Symposium on Sterol Structure, *J. Soc. Chem. Ind.*, **52**, 10 (1933).

Stereochemistry *

In cholestane (LXXIV), the parent hydrocarbon of cholesterol and dihydrocholesterol, there are centers of asymmetry at C₅, C₈, C₉, C₁₀, C₁₃, C₁₄, C₁₇, and C₂₀. The number of possible stereoisomers is accordingly 2⁸, or 256. With an hydroxyl group at C₃ the possible isomers are increased to 512. Coprostanane (LXXV) is one of the stereoisomers of cholestane, differing only in the spatial arrangement of the C₅—H.



group are derivatives of these two hydrocarbons, and it would be valuable to have precise structures for both. Unfortunately, it is possible to portray only the probable structures and indicate the interrelationship.

The space models for the cholestane type (Fig. 1) and the coprostanane type (Fig. 2) are suggested by similar representations by Ruzicka.⁵⁹

* The several aspects of stereochemistry are considered in Chap. 3, p. 247.

⁵⁹ Ruzicka, Furter, and Thomann, *Helv. Chim. Acta*, **16**, 331 (1933).

The models are built up from the assumption that the $C_{10}-CH_3$ (R) group in both structures projects out from the plane of the paper. The size of the balls does not show the probable atomic size, nor are the interatomic distances correctly represented; besides these defects, the position in space of the angular substituents and the relationship of one portion of the molecule to another may be entirely different from those shown. Nevertheless, the models are helpful in understanding the adjustments that probably occur in the change from one type to the other. Assuming that the $C_{10}-CH_3$ comes forward, then in the cholestane type the C_5-H goes into the plane of the paper, and in the

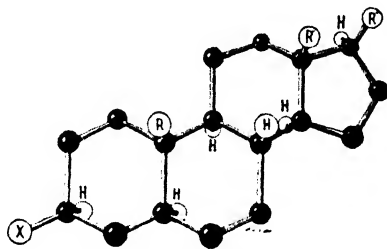


FIG. 1.—Cholestane type

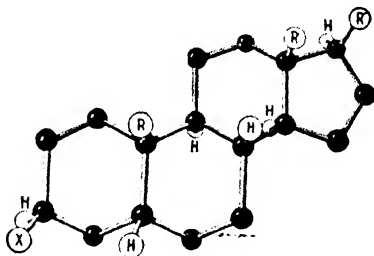


FIG. 2.—Coprostane type

(The illustrations are reproductions of space models of the two types. The position in space of the atoms is shown by the size and the shading of the balls that are used to represent the various atoms or groups. The atoms nearest the eye are shown by the largest balls, and, where these represent carbon atoms, by full intensity of black. The atoms in planes below this are represented by smaller balls and by decreased intensity of black. The circles marked "X" represent any group attached at C_3 .)

coprostane type it comes out from the plane of the paper. The spatial adjustments in the configuration of rings A and B are such that in cholestane these two rings are "chair types"; in coprostane, "bed types" (p. 49). From the models it can be seen that a substituent at C_3 does not occupy exactly the same relative position either to the C_5-H or to $C_{10}-CH_3$ in both types. Aside from these differences the two molecules appear to be the same. The evidence presented below does

not disclose any discrepancies in these representations, but the methods used to obtain it are not completely satisfactory.

For representations on a plane surface, dotted and solid lines are employed to indicate the spatial configuration. As has been mentioned previously, the dotted lines indicate bonds going into the plane of the paper; the solid lines, bonds lying in or coming out of the plane of the paper. The use of such lines (as is shown in structures LXXIVa and LXXVa) for an entire structure is too cumbersome for common use, and in practice only selected portions of the molecule are so represented. The method has the disadvantage that a solid line is the normal way of representing a linkage, and where the spatial configuration is unknown, the implied structure may be erroneous.

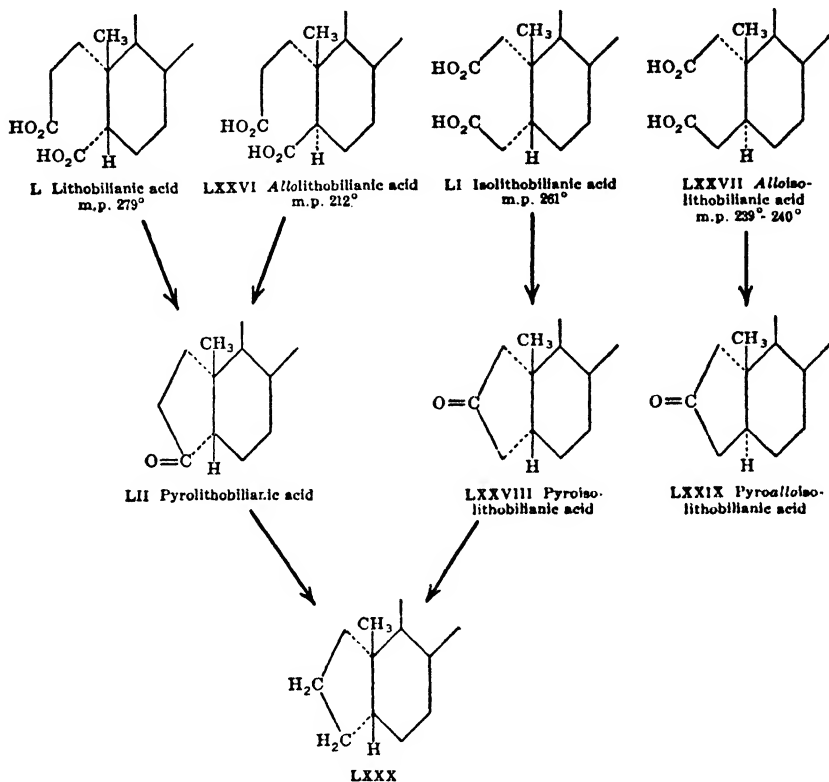
Spatial Isomerism of the Nuclear Rings. The experimental evidence in support of the structures of cholestane and coprostane has been obtained largely from the chemical behavior of degradation products of the bile acids, and from physical measurements on the hydrocarbons themselves.

Rings A/B. Windaus⁶⁰ has studied the behavior of the four lithobilianic acids when subjected to thermal decomposition. Lithobilianic acid (L) and *allolithobilianic* acid (LXXVI) give the same pyro acid (LII); *isolithobilianic* acid (LI) and *alloisolithobilianic* acid (LXXVII) give two different pyro acids, LXXVIII and LXXIX. Clemmensen reduction of pyrolithobilianic acid and of pyroisolithobilianic acid gives the same desoxo compound (LXXX).^{*} Lithobilianic acid and *isolithobilianic* acid must have the C₅-H and the C₁₀-CH₃ in the same relationship, since both are produced from lithocholic acid and both can be transformed to the same end product. *Allolithobilianic* acid and *alloisolithobilianic* acid give different pyro acids and desoxypyroacids. Obviously, *allolithobilianic* acid must have undergone a rearrangement in the thermal treatment, and the same arguments apply here as were used previously in the case of the acid C₁₃H₂₀O₆ (LXVIII) (p. 1248), that a rearrangement from a *trans* to a *cis* structure occurs. Since the lithobilianic acids may be regarded as degradation products of coprostane in which ring A has been opened, and the *allolithobilianic* acids as degradation products of cholestane, it follows that rings A B have a *cis* relationship in coprostane and a *trans* relationship in cholestane. Similar results have been obtained by Lettré⁴⁰ with the corresponding dicarboxylic acids from dihydrocholesterol and copro-

⁶⁰ Windaus, *Ann.*, **447**, 240 (1926), and reference 57.

^{*} *Allolithobilianic* acid and *alloisolithobilianic* acid may be prepared from hyodesoxycholic (3,6-dihydroxycholanic) acid or from cholesterol. The transformation is discussed later (p. 1308).

sterol. Not only do these transformations establish the relationship of rings A/B, but also, they show that aside from these rings the spatial configuration of the two types is the same.



Ruzicka⁶¹ has examined the physical constants of cholestane (LXXIV) and coprostanane (LXXV), and compared them with the known examples of the *cis* and *trans* decalins. Cholestane was found to have a lower density, higher molecular refraction, and higher melting point than coprostanane. Reasoning from the decalins (p. 402), the relationship of rings A/B is *trans* in cholestane and *cis* in coprostanane.

In addition to the differences in the physical properties in the two structures, there are chemical dissimilarities. When the 3-keto compounds of the cholestane type are oxidized to acids, ring opening takes place principally between C₂—C₃, while with coprostanane derivatives, opening of ring A occurs chiefly at C₃—C₄.⁶² The dicarboxylic acids

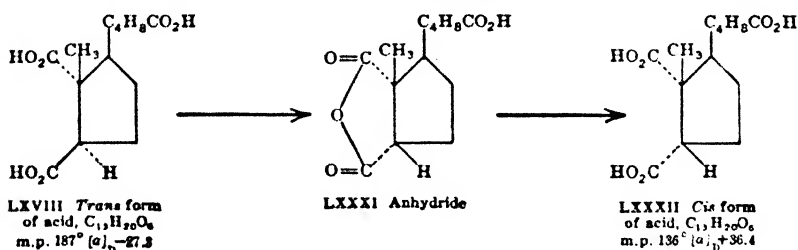
⁶¹ Ruzicka, Furter, and Thomann, *Helv. Chim. Acta*, **16**, 327 (1933).

⁶² Wieland, Dane, and Martius, *Z. physiol. Chem.*, **215**, 15 (1933).

formed by opening the ring between C₂—C₃ are more soluble than those resulting from the cleavage of the C₃—C₄ bond. On treating the 3-keto compounds with bromine, substitution takes place predominantly at C₄ with the coprostane structure, and at C₂ with the cholestane structure.⁶³ These reactions suggest that enolization is primarily from C₄ to C₃ in one case, and C₂ to C₃ in the other.

Rings B/C. The x-ray and surface film measurements of Bernal¹² show that the molecules of the sterols must be flat, as in paraffin hydrocarbons with methyl side chains. Models of the sterol molecules in which rings B/C are *trans* are flat, in agreement with the physical measurements, while a *cis* structure at this point gives a bowed-in, or condensed model. Another positive argument for such a configuration comes from the work of Wieland.⁶⁴ After 7,12-diketocholanic acid is heated for ten hours with dilute alkali, it can be recovered unchanged. Since the neighboring center of asymmetry to C₁₂ carries a methyl group, rearrangement at this point is impossible, and the absence of rearrangement must mean that the C₈—H is in the stable *trans* configuration with respect to the C₉—H. This is in accord with Hückel's⁶⁵ experience that only *cis*-decalones rearrange into *trans* when treated with alkali. For convenience rather than with reason, the C₉—H is usually represented as being *trans* to the C₁₀—CH₃, and the practice is frequently bulwarked by the argument of steric hindrance.

Rings C/D. The behavior of the acid C₁₃H₂₀O₆ (LXVIII) on thermal decomposition furnishes the evidence for a *trans* relationship of the C₁₃—CH₃ and the C₁₄—H.* The structural representation of this



transformation, which was not given previously, is cited here. In the production of the anhydride, LXXXI, a rearrangement occurs, and on saponification the lower-melting *cis* form of the acid (LXXXII) results.

⁶³ Summary: Butenandt, Schramm, Wolff, and Kudszus, *Ber.*, **69**, 2779 (1936). Cf. Ruzicka, Bosshard, Fischer, and Wirz, *Helv. Chim. Acta*, **19**, 1147 (1936).

⁶⁴ Wieland and Wiedersheim, *Z. physiol. Chem.*, **186**, 232 (1930).

⁶⁵ Hückel, *Ann.*, **441**, 1 (1925). Cf. Windaus, Hückel, and Revere, *Ber.*, **56**, 91 (1923); Linstead and Meade, *J. Chem. Soc.*, 935 (1934); Cook and Linstead, *ibid.*, 946 (1934); Barrett and Linstead, *ibid.*, 436 (1935).

* For an argument against a *trans* configuration, see Peak, *Nature*, **140**, 280 (1937).

The Side Chain at C₁₇. The ready formation of dehydronorcholene (XIV) from 12-ketocholanic acid (XIII) has been used by Wieland as an argument that the side chain at C₁₇ occupies a *trans* position with respect to the C₁₃—CH₃. Consideration of space models hardly confirms this contention, and Ruzicka⁶⁶ has suggested that a *cis* configuration is a better representation. In Figs. 1 and 2, this side chain is shown in the *cis* configuration, but without convincing evidence the matter must be left open.

Spatial Isomerism of the Hydroxyl Groups. There are a number of methods used to describe the steric position of the ring hydroxyl groups, particularly that of the C₃—OH. The prefix “*epi*” is used in the sterol series as a means of indicating a configuration different from the “normal” type found in nature. In the bile acid series the configuration analogous to the *epi* form is spoken of as an α type, while the normal configuration is a β type. The burden of evidence indicates that the normal hydroxyl group is in a *cis*-position with respect to the angular methyl group at C₁₀ and that the *epi*-modification is *trans* to C₁₀—CH₃. Wherever the spatial configuration of the C₃—OH is described in this discussion, the hydroxyl groups are referred to the C₁₀ angular methyl group and the spatial configuration is indicated by a parenthetical designation; for example, dihydrocholesterol (XX) is described as 3(*cis*)-hydroxycholestane.*

Ruzicka has introduced the practice of referring the C₃—OH to the hydrogen at C₅. This convention is somewhat unsatisfactory, since in many compounds there is no hydrogen at C₅. In cases of this sort Ruzicka ascribes to the hydroxyl the same position as that which it occupies in the corresponding saturated compound. Thus the hydroxyl group in both dihydrocholesterol and cholesterol is described by Ruzicka

⁶⁶ Ruzicka, Goldberg, and Wirz, *Helv. Chim. Acta*, **18**, 61 (1935).

* In the appendix to his revised monograph, p. 398, Fieser discusses the stereochemical nomenclature. He points out that the angular methyl groups are not wholly satisfactory reference points, since they do not always have the same position in space. For example, lumisterol (p. 1290) is a compound in which the C₁₀—CH₃ has an opposite configuration to that of cholesterol. This is not a serious objection, however, since the configuration of the angular methyl group is shown by the parent name, e.g., cholestane and lumistane. Fieser also objects to the use of *cis* and *trans* to describe the position of the C₃—OH group with respect to either the C₅—H or the C₁₀—CH₃, since these terms are appropriate only for the groupings attached to adjacent carbon atoms. As a substitute for the terms *cis* and *trans* he has suggested that the configuration be indicated by a parenthetical α or β . By this system dihydrocholesterol would be named 3(β)-hydroxycholestane. Fieser regards this as desirable, since dihydrocholesterol is also known as β -cholestanol and adoption of the proposed notation would be compatible with the older notation of the bile acids. On the other hand, the Greek letters α and β are also used to describe different crystalline modifications, e.g., α - and β -progesterone (p. 1369), and as temporary designations for isomeric compounds in which the configuration has not been examined.

as 3(*trans*)-hydroxy, although in cholesterol the reference hydrogen at C₅ is absent.*

In this discussion the groups attached at positions other than C₃ are likewise referred to the nearest angular methyl group and their steric relations are described by the term *cis* or *trans*. The use of these terms to relate groupings several atoms apart is somewhat more liberal than precedent justifies, but by referring all groups to the nearest angular methyl group, a consistent practice is possible and adjustment can easily be made when a more rational nomenclature is developed.

The C₃—OH. Vavon and Jakubowicz⁶⁷ have studied the epimeric modifications, dihydrocholesterol (XXIX) and *epidi*hydrocholesterol (LXXXIV). Catalytic hydrogenation of cholesterol or cholestanone † (LXXXIII) in neutral solvents gives dihydrocholesterol,‡ while its epimer is formed from cholestanone by hydrogenation in acid media (acetic acid and hydrobromic acid or butyl ether and hydrobromic acid). Applying the rule of v. Auwers-Skita⁶⁸ that neutral media favor the formation of *trans* forms and acid media lead to *cis* structures, dihydrocholesterol is 3(*trans*)-hydroxycholestane and *epidi*hydrocholesterol is 3(*cis*)-hydroxycholestane, the hydroxyl being referred to the C₅—H in both instances. Since this C₅—H is *trans* to the C₁₀—CH₃, the designations become 3(*cis*)-hydroxycholestane for dihydrocholesterol and 3(*trans*)-hydroxycholestane for the *epi* compound, when the angular methyl group is used as the reference point.

This work of Vavon and Jakubowicz has been extended by Grasshof⁶⁹ and Ruzicka^{37, 70} who, in addition to studying dihydrocholesterol and *epidi*hydrocholesterol, prepared coprosterol (LXXXVI) and *epicopro*sterol (LXXXVII) by the catalytic hydrogenation of coprostanone (LXXXV). The latter, in turn, was obtained by the catalytic hydrogenation of cholestenone (XXIV). Applying v. Auwers-Skita's rule to the formation of coprosterol and its epimer, the hydroxyl is *cis* in the former and *trans* in the latter. Reference is made to the C₅—H, of course, but since this is *cis* to the C₁₀—CH₃, the spatial relationship is the same to both points of reference. Apparently the rule of v. Auwers-

* In his current publications, Ruzicka is abandoning this practice and is differentiating between the two forms by using the prefix "epi" for the configuration differing from the normal.

⁶⁷ Vavon and Jakubowicz, *Bull. soc. chim.*, **53**, 581 (1933).

† For preparative details see Bruce, "Organic Syntheses," John Wiley and Sons, New York (1937), Vol. 17, p. 43.

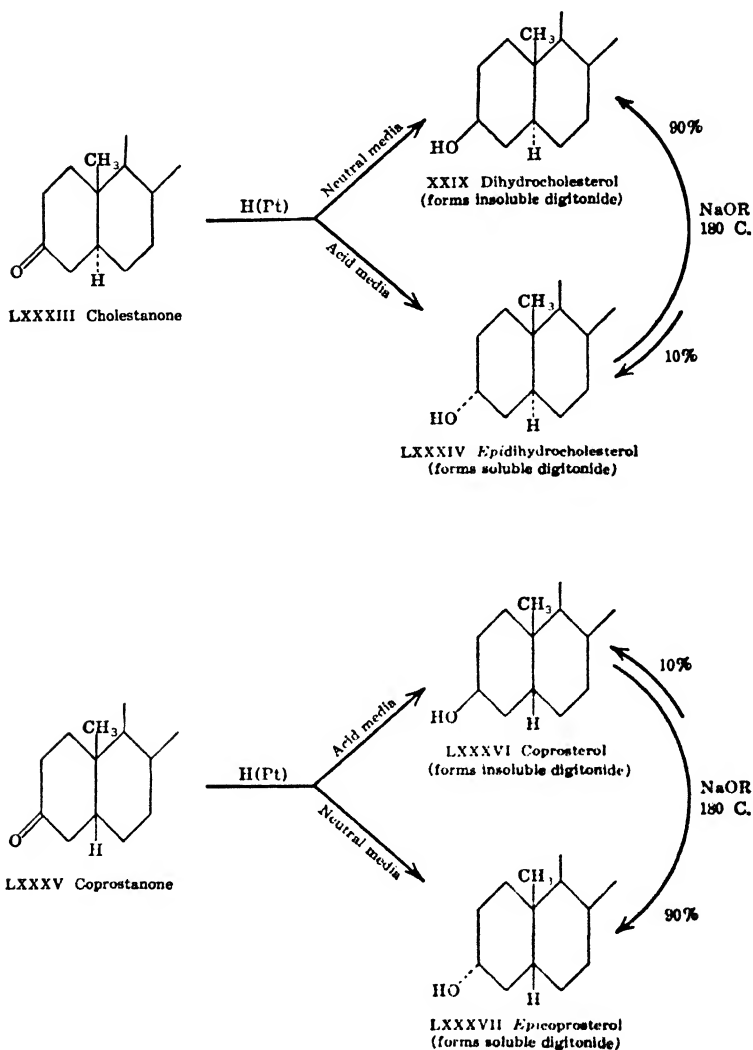
‡ For preparative details see Bruce and Ralls, "Organic Syntheses," John Wiley and Sons, New York (1937), Vol. 17, p. 45.

⁶⁸ v. Auwers, *Ann.*, **420**, 91 (1920); Skita, *Ber.*, **53**, 1792 (1920).

⁶⁹ Grasshof, *Z. physiol. Chem.*, **225**, 197 (1934).

⁷⁰ Ruzicka, *Helv. Chim. Acta*, **19E**, 90 (1936).

Skita is generally valid, although Ruzicka noted a definite exception to it in the production of coprostanone from cholestanone.



Sodium ethoxide (or sodium and xylene) produces epimerization of the hydroxylated derivatives when they are heated together in anhydrous media at 180° . An equilibrium mixture results from such treatment. In this mixture the *trans* form (referred to the C_5-H) predominates in a ratio of about 9 : 1. The product of such a rearrangement can be resolved through the aid of the insoluble addition compound that the

saponin digitonin (p. 1346) forms with neutral compounds of this group in which the C_3 —OH stands in a *cis* relationship to the C_{10} — CH_3 . Usually the precipitation is carried out in 90 or 95 per cent alcohol, but occasionally a 50 per cent methanol-water solution is used.⁷¹ Where the C_{17} side chain differs greatly from the normal, as for example in a compound containing a diphenylethylene group fused to the side chain, precipitation is prevented even though the steric position of the C_3 —OH group is correct.⁷² Other compounds in which the C_3 —OH group is *cis* to the C_{10} — CH_3 group but which fail to give insoluble digitonides are cholestanetriol (XXI),⁷² and the so-called isopregnanolones and related compounds (p. 1369). On the other hand, a few ketones in which the C_3 —OH group is blocked or absent, such as 3-acetoxy-20-ketoallopregnane (allopregnanol-3-one-20-acetate) and 3,20-diketoallopregnane, form sparingly soluble digitonides, but the rate of formation is slow.⁷³ That it is not absolutely essential for a C_{10} — CH_3 group to be present for the formation of insoluble digitonides is shown by the fact that neoergosterol⁷⁴ (see below) and one of the hexahydroestrone⁷⁵ are precipitated by digitonin.* Yet if the methyl group is present in an *epi* configuration, then precipitation does not occur, e.g., lumisterol and pyrocalciferol (p. 1298). It is obviously difficult to give a rigid interpretation, but the formation of an insoluble digitonide is usually taken as evidence of a *cis* configuration of the C_3 —OH to the C_{10} — CH_3 group;⁷⁶ failure of a precipitate to form may or may not indicate a *trans* configuration.⁷⁷

W. Stoll⁷⁷ has suggested another means of establishing an *epi* configuration. The *p*-toluenesulfonates of the normal saturated sterols react slowly when boiled with methyl alcohol to form methyl ethers, but the *p*-toluenesulfonates of the epimers are converted into unsaturated compounds by the same treatment. It is difficult to evaluate this method because it has been applied to relatively few compounds.

Walden Inversion of the C_3 —OH. When cholesterol (II) is treated with phosphorus pentachloride or thionyl chloride, the hydroxyl is

⁷¹ Windaus, *Ber.*, **43**, 238 (1909); Reichstein, *Helv. Chim. Acta*, **19**, 406 (1936).

⁷² Fernholz, *Z. physiol. Chem.*, **232**, 97 (1935).

⁷³ Butenandt and Mamoli, *Ber.*, **68**, 1847 (1935).

⁷⁴ Bonstedt, *Z. physiol. Chem.*, **185**, 168 (1929).

⁷⁵ Dirscherl, *ibid.*, **239**, 53 (1936).

* As is shown later (p. 1363) the reduction of the female sex hormone, estrone, gives two isomeric α - and β -estradiols in which there is isomerism about C_{17} . Of the two isomers only α -estradiol forms an insoluble digitonide, but the rate of precipitation is slow in comparison with that of the sterols. It is probable that digitonide formation takes place with the C_{17} —OH, but at present it is impossible to give an interpretation [Wintersteiner, *J. Am. Chem. Soc.*, **59**, 765 (1937)].

⁷⁶ Schoenheimer and Evans, *J. Biol. Chem.*, **114**, 567 (1936).

⁷⁷ Stoll, *Z. physiol. Chem.*, **245**, 1 (1937).

replaced with chlorine. Regeneration of the hydroxyl by treatment with potassium acetate and saponification returns the original cholesterol. If, however, the cholesteryl chloride is catalytically hydrogenated and the chlorine then replaced with hydroxyl, *epidihydrocholesterol* is obtained rather than dihydrocholesterol, and, obviously, at some stage of this transformation there is a Walden rearrangement (pp. 197, 1844) about C₃.⁷⁸ The two chlorinating reagents behave differently with dihydrocholesterol and its epimer. Phosphorus pentachloride with dihydrocholesterol gives the so-called β -cholestyl chloride, m.p. 102°, ⁷⁹ but thionyl chloride produces α -cholestyl chloride, m.p. 112°. ⁸⁰ With *epidihydrocholesterol* the two reagents produce the α - and β -compounds, respectively. Although the mechanism of these changes is not understood, the rearrangements are of value in the production of the sex hormone, androsterone. ⁷⁸

The spatial position of the hydroxyl group at C₃ influences its physical properties and chemical reactivity. Reindel and Niederländer⁸¹ have compared the melting points of a large number of the saturated stereoisomers. In both the cholestane and the coprostane series, the member of an epimeric pair that gives an insoluble digitonide always has the lower melting point. Interpreting this as above, those saturated compounds in which the C₃—OH is *cis* to the C₁₀—CH₃ have lower melting points than the epimers. Vavon and Jakubowicz⁶⁷ observed that the *trans* form (to C₅) is esterified more easily and dehydrated less readily than the *cis* modification.

Biochemically the spatial position of the C₃—OH is of great importance, although only in the case of the sex hormones and possibly of the cardiac principles is it possible to correlate stereochemistry and physiological activity. Indeed, so clear cut are the physiological effects of epimerizing the C₃—OH in the case of the male sex hormones, that the bioassay may be used as a means of determining the structure.

The C₇—OH. Where the hydroxyl group is attached at any position other than C₃, it is difficult to obtain good evidence of the configuration aside from that afforded by catalytic hydrogenation. In the case of certain of the bile acids, however, there are hydroxyl groups at both C₃ and C₇. Here it is possible by hypobromite oxidation to open ring A to a 7-hydroxylithobilianic acid and to study the ease of lactone formation between the carboxyl at C₅ and the C₇—OH. Should a lactone

⁷⁸ Marker, *J. Am. Chem. Soc.*, **57**, 1755 (1935); Marker, Whitmore, and Kamm, *ibid.*, **57**, 2358 (1935). (Cf. Ruzicka *et al.*, reference 80, and Lettré, reference 83.)

⁷⁹ Diels and Linn, *Ber.*, **41**, 548 (1908); Ruzicka, Goldberg, and Brüngger, *Helv. Chim. Acta*, **17**, 1389 (1934).

⁸⁰ Ruzicka, Wirz, and Meyer, *ibid.*, **18**, 998 (1935).

⁸¹ Reindel and Niederländer, *Ann.*, **522**, 218 (1936).

result, then, according to the rule of Alder-Stein,⁸² a *cis* arrangement of the carboxyl and hydroxyl is present. Application of this method by Lettré⁸³ to chenodesoxycholic (3,7-dihydroxycholanic) acid and to cholic acid (III) shows that the C₇—OH is *trans* to the C₁₀—CH₃; in both of these cases lactones were formed.

Structure and Optical Rotation. Relatively little work has been done on the correlation of structure and optical rotation (p. 1803). From the molecular rotations of various pairs of compounds Callow and Young⁸⁴ have noted that epimerization of the C₃—OH group from the *cis* position with respect to the C₁₀—CH₃ group to a *trans* position is accompanied by a shift of rotation to the right. This increase of *d*-rotation is seen from the data given in the tables in the following discussion, where, for comparison, the specific rotations of isomers serve as well as the molecular rotations. There are not enough cases of inversion of the hydroxyl group at C₄, C₅, and C₁₇ to permit conclusive generalizations to be made for these positions, but the introduction of a double bond into the molecule alters the *d*-rotation as follows: Compounds with unsaturation at Δ¹, Δ⁵, and Δ²² show a marked decrease in *d*-rotation; at Δ¹⁴, a small decrease; at Δ⁷, an irregular effect; at Δ¹⁴, a small increase; and at Δ⁴, a marked increase. Reduction of a C₁₇ carbonyl group to carbinol decreases the *d*-rotation slightly.

Lettré⁸⁵ has extended this work by studying the molecular rotations of neoergosterol (LXXXVIII) and *epi*neoergosterol (LXXXIX) and their derivatives. In these sterols there are no centers of asymmetry in ring B, and the rotation is due entirely to the effects of asymmetric carbon atoms, C₃, C₁₂, C₁₄, C₁₇, and the side chain. The asymmetric center at C₃ is so far removed from the other centers that the total rotation may be split up into two parts—part B, due to C₃, and part A, due to the rest of the molecule. On calculating the values for A and B in neoergosterol and *epi*neoergosterol, it is apparent that B (C₃) has a negative value in neoergosterol, but is positive in *epi*neoergosterol. The

$$\text{Neoergosterol: } [\alpha]_D - 11 \cdot [M]_D = -41.8 = A - B = 31.2 - 73$$

$$\text{Epi}neoergosterol: [\alpha]_D + 27.4 \cdot [M]_D = +104.2 = A + B = 31.2 + 73$$

same relationship applies to derivatives of the two sterols. Because of the sign of the rotation, neoergosterol may be regarded as a derivative of (–)-*ac*-tetrahydro-β-naphthol (XC) and *epi*neoergosterol of (+)-*ac*-

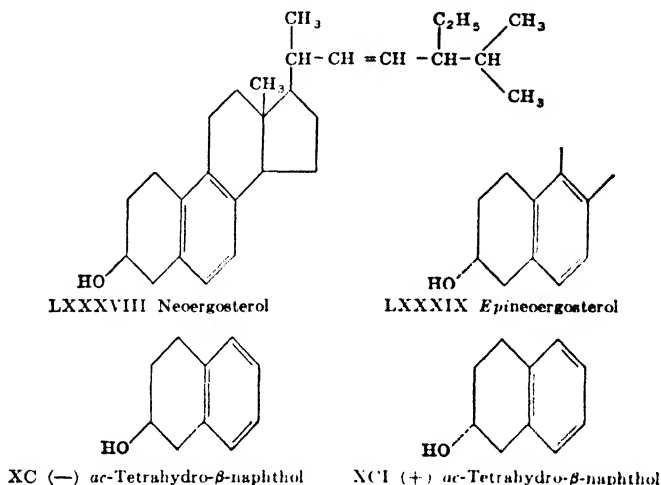
⁸² Alder and Stein, *Ann.*, **504**, 229 (1933).

⁸³ Lettré, *Ber.*, **68**, 766 (1935).

⁸⁴ Callow and Young, *Proc. Roy. Soc. (London)*, **A157**, 194 (1936).

⁸⁵ Lettré, *Ber.*, **70**, 450 (1937).

tetrahydro- β -naphthol (XCI) ($[M]_D = \pm 99.5$).⁸⁶ Since neoergosterol or epineoergosterol can be correlated with the other compounds of the cyclopentanoperhydrophenanthrene group containing a C_3-OH group, all these compounds are related to the *ac*-tetrahydro- β -naphthols. It is possible that further study of the relationship of structure and



optical rotation may lead to generalizations that will serve to check constitutional formulas based on chemical reactions.

THE STEROLS⁸⁷

Definition and Occurrence. The sterols may be defined as the saturated and unsaturated alcohols derived from cyclopentanoperhydrophenanthrene. In nature they are widely distributed, both free and combined as esters or glycosides (p. 1454). Esters of the sterols with fatty acids are common to both animal and plant life, but the glycosides, the so-called sterolins, occur only in plants. For classification, the sterols are divided on the basis of occurrence into zoosterols (animal sterols), phytosterols (plant sterols), and mycosterols (sterols of yeast and fungi).

Isolation. The sterols are the principal constituents of the non-saponifiable portions of fat extracts. They are obtained as well-crystallized substances with a waxy feel. From alcohol the crystals separate out with a molecule of water; from ether the anhydrous form is ob-

⁸⁶ Pickard and Kenyon, *J. Chem. Soc.*, **101**, 1427 (1912).

⁸⁷ Books: Fieser; Lettré and Inhoffen; "Biochemisches Handlexikon," Springer, Berlin (1911), III, 268; (1914), VIII, 473; (1923), X, 156; "Ann. Rev. Biochem.," **I**, 105 (1932); III, 87 (1934); VI, 139 (1937). Reviews: Heilbron, *J. Soc. Chem. Ind.*, **55**, 129T (1936). For physiology of the sterols, see Bills, *Physiol. Rev.*, **15**, 1 (1935).

tained. Since more than one sterol is generally present in any natural product, separation is often rather difficult. The occurrence of mixed crystals and of molecular compounds sometimes makes purification of the free alcohol by recrystallization impractical. Advantage may then be taken of the differential solubilities of derivatives, such as the dibromides or the digitonides, from which the sterol may be regenerated. With the dibromides the regeneration is effected by treatment with zinc and acetic acid⁸⁸ or by heating with an alcoholic solution of sodium iodide.⁸⁹ The digitonides are split by treating with pyridine,⁹⁰ by extracting with boiling xylene, or by acetylating.⁹¹

Because of the difficulty in obtaining pure compounds, many of the analyses reported in the literature are erroneous. Even though a pure compound is available for analysis, the results may be hard to interpret. For this reason, analyses must be conducted on the free sterol, and on its acetate⁹² and other esters, such as the dinitrobenzoate.⁹³ The properties of a number of the more important sterols are given in Table II (pp. 1264-65).

Nomenclature. Most of the sterols are systematically named by referring them to the two stereoisomeric hydrocarbons, cholestane and coprostane (p. 1251), the spatial configuration of the hydroxyl group being described by a suitable prefix. The use of the terms *cis* and *trans* to describe the spatial relationship of the hydroxyl groups is confusing unless the reference point is given, and even then may be wholly erroneous. The prefix "epi" is the safest method of indicating the configuration, but it, in turn, lacks precision. With the unsaturated sterols, particularly those with unsaturation at C₅, there is a problem of reference compound. It seems best to refer them all to the stable cholestane configuration, rather than to relate some to this compound and others to coprostane.⁹⁴

General Reactions of the Sterols

Most of the natural sterols have the general architecture given in structure I. As this structure shows, the sterol molecule is hydroxylated

⁸⁸ Windaus, *Ber.*, **39**, 518 (1906); Fernholz, *Ann.*, **507**, 122 (1933).

⁸⁹ Schoenheimer, *J. Biol. Chem.*, **110**, 461 (1935).

⁹⁰ Schoenheimer and Dam, *Z. physiol. Chem.*, **215**, 59 (1933). This is the preferred method.

⁹¹ Windaus, *ibid.*, **65**, 110 (1910).

⁹² Sandqvist and Gorton, *Ber.*, **63**, 1935 (1930); Sandqvist and Bengtsson, *Ber.*, **64**, 2167 (1931).

⁹³ Windaus, Werder, and Gschaidler, *Ber.*, **65**, 1006 (1932).

⁹⁴ Cf. Rosenheim and King, "Ann. Rev. Biochem.," vol. III, p. 87 (1934), for proposal for nomenclature.

TABLE II
PRINCIPAL NATURAL AND DERIVED STEROLS*

Sterol †	Structure ‡	Formula	Δ §	Insol- uble Digi- tonide	M.P. ° C.	$[\alpha]_D$ (CHCl ₃)	Source or Derivation
Zoosterols							
<i>Allopregnanediol</i> (Nat.)	3(<i>trans</i>), 20-Dihydroxyallopreg- nane	C ₂₇ H ₄₆ O ₂	0	—	248 c.	Pregnancy urine
<i>Allopregnanediol</i> (Syn.)	3(<i>cis</i>), 20-Dihydroxyallopreg- nane	C ₂₇ H ₄₆ O ₂	0	+	195–196	<i>Allopregnanedione</i>
<i>Pregnanediol</i>	3(<i>trans</i>), 20-Dihydroxypregnane	C ₂₇ H ₄₆ O ₂	0	—	237 c.	Pregnancy urine
<i>7-Dehydrocholesterol</i>	3(<i>cis</i>)-Hydroxy-5,7-cholestadi- ene	C ₂₇ H ₄₄ O	2	+	142–143.5	–113.6	Cholesterol
<i>Allocholesterol</i>	3(<i>cis</i>)-Hydroxy-4-cholestene	C ₂₇ H ₄₄ O	1	+	132	+43.7 (C ₆ H ₆)	Δ^4 -Cholestenone
<i>Epiallocholesterol</i>	3(<i>trans</i>)-Hydroxy-4-cholestene	C ₂₇ H ₄₄ O	1	—	84	+120.8 (C ₆ H ₆)	Δ^4 -Cholestenone
<i>Cholesterol</i>	3(<i>cis</i>)-Hydroxy-5-cholestene	C ₂₇ H ₄₆ O	1	+	150–151	–39.5	All animal tissues, esp. brain and spinal cord; gallstones
<i>Epicholesterol</i>	3(<i>trans</i>)-Hydroxy-5-cholestene	C ₂₇ H ₄₆ O	1	—	141	–35	Cholesteryl chloride
<i>Cholesterol</i>	3(<i>trans</i>)-Hydroxy-5-cholestene	C ₂₇ H ₄₆ O	0	—	74–75	+23.9	Cholesteryl <i>p</i> -toluenesul- fonate
<i>7-Hydroxycholesterol</i>	3(<i>cis</i>), 7-Dihydroxy-5-cholestene	C ₂₇ H ₄₆ O ₂	1	+	172	+94.2	Cholesterol
<i>β-7-Hydroxycholesterol</i>	3(<i>cis</i>), 7-Dihydroxy-5-choles- tene	C ₂₇ H ₄₆ O ₂	1	+	184–185	–86.4	Cholesterol
<i>Dihydrocholesterol</i>	3(<i>cis</i>)-Hydroxycholestane	C ₂₇ H ₄₈ O	0	+	140–141	+23.5	Cholesterol, feces
<i>Epidihydrocholesterol</i>	3(<i>trans</i>)-Hydroxycholestane	C ₂₇ H ₄₈ O	0	—	183–184	+33.9	Cholesterol
<i>Coprosterol</i>	3(<i>cis</i>)-Hydroxycoprostanane	C ₂₇ H ₄₈ O	0	+	100–101 c.	+24.7	Feces, cholesterol
<i>Epicooprosterol</i>	3(<i>trans</i>)-Hydroxycoprostanane	C ₂₇ H ₄₈ O	0	—	111 c.	+31.5	Coprostanone
<i>Oosterol</i>	C ₂₇ H ₄₈ O		2	+	142–143	–43.6	Oysters, clams

Phytosterols

α -Spinasterol	3(cis)-Hydroxy-24-ethyl-5,7,22-cholestatriene	$C_{27}H_{46}O$	2	+	172 c.	-3.7	Spinach, senega root
7-Dehydrostigmasterol		$C_{27}H_{44}O$	3	+	154	-113.15	Stigmasterol
Fucosterol		$C_{27}H_{46}O$	2	+	124	-38.4	Bladder wrack
α -Sitosterol		$C_{27}H_{46}O$	2	+	166	-1.7	Wheat germ oil
Stigmasterol		$C_{27}H_{44}O$	2	+	169-170	-45	Soy and calabar beans
Cincho		$C_{27}H_{46}O$	1	+	140-141	-38	Cinchona bark
β -Sitosterol		$C_{27}H_{46}O$	1	+	136-137	-34.2	"Talloi," sarsaparilla root
γ -Sitosterol		$C_{27}H_{46}O$	1	+	145-146	-42	Chief sterol of plants
γ -Sitosterol		$C_{27}H_{46}O$	0	+	144-145 c.	+27.8	γ -Sitosterol
Stigmasterol		$C_{27}H_{44}O$	0	+	137	+24.8	Stigmasterol
<i>Epistigmasterol</i>		$C_{27}H_{44}O$	0	-	200	+25	Stigmasterol
α -Sitosterol		$C_{27}H_{46}O$	1	+	156	+3.5	Wheat germ oil

Mycosterols

Zymosterol	3(cis)-Hydroxy-24-methyl-5,7,22-cholestatriene	$C_{27}H_{44}O$	2	+	108-110	+47.3	Yeast
Ergosterol		$C_{27}H_{44}O$	3	+	163	-132	Ergot, yeast
22-Dihydroergosterol		$C_{27}H_{46}O$	2	..	152-153	-109	Ergosterol
Ergostanol		$C_{27}H_{46}O$	0	+	150-151	+15.3	Ergosterol

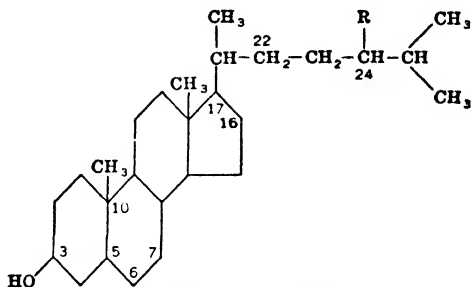
* Sources of data other than those cited in the text: Cholesterol, Anderson, *J. Biol. Chem.*, **71**, 407 (1926); Dihydrocholesterol, Anderson, *ibid.*, **71**, 411 (1926); Epidihydrocholesterol, Windaus and Ubrigg, *Ber.*, **47**, 2386 (1914); Epicocholesterol, Dodge, *J. Chem. Soc.*, **95**, 645 (1909); Stigmasterol, Windaus and Haub, *Ber.*, **39**, 4373 (1906); β -Sitosterol, Simpson and Williams, *J. Chem. Soc.*, **733** (1937); Zymosterol, Wieland and Asano, *Ann.*, **473**, 303 (1929); Ergosterol, Bills and Honeywell, *J. Biol. Chem.*, **80**, 23 (1928).

† In the older literature, the following terms have been used: β -cholestanol for dihydrocholesterol; γ -cholestanol for a molecular compound of dihydrocholesterol, and γ -epicocholesterol; δ -cholestanol for epidihydrocholesterol.

‡ The position of the C-3-OH is referred to the C₁₀-CH₃ in all cases. The use of the symbol Δ to indicate unsaturation has been avoided; this is in keeping with the current practice in *Chemical Abstracts* of omitting this symbol where it is not indispensable.

§ Double bonds.

at C_3 , is unsaturated in ring B, and has at C_{17} a secondary isoöctyl side chain, which is often substituted at C_{24} with ethyl or methyl groups, and which may be unsaturated at C_{22} . There are a number of poorly investigated sterols, and their final formulation may change the picture somewhat; but for the present the general reactions of the sterols can be discussed by considering the several characteristics individually in relation to structure I.



I Ring system of the sterols

(The side chains shown are usually present. R of C_{17} side chain may be H or alkyl. Unsaturation is frequently present at C_5 . The C_3 -OH may be *cis* or *trans* to C_{10} -CH₃).

The C_3 -OH. The hydroxyl group at C_3 is generally the only alcoholic function that is present in a sterol. The position of this group doubtless shows something of the biogenesis of the sterols, but no interpretation can be given at the present time. In addition to the usual reactions of a secondary hydroxyl, the C_3 -OH undergoes stereochemical transformations that are conditioned by the structure of the molecule and the reagents used. The Walden inversion that takes place when the saturated sterols are treated with chlorinating reagents and regenerated has been discussed (p. 1259). A similar kind of rearrangement is known for the unsaturated sterols.

Cholesteryl *p*-toluenesulfonate and methanol react smoothly to give two different cholesteryl methyl ethers.⁹⁵ With methanol alone the well-known *l*-ether,⁹⁶ m.p. 84° , $[\alpha]_D - 42$, is obtained; but with methanol and anhydrous potassium acetate, a *d*-ether, m.p. 79° , $[\alpha]_D + 51.8$ results. The same products are formed if cholesteryl chloride is treated with methanol or with methanol-potassium acetate.⁹⁷ The *d*-ether may be a derivative of *i*-cholesterol or allo-cholesterol* (see below). With aqueous potassium hydroxide-acetate,

⁹⁵ Stoll, *Z. physiol. Chem.*, **207**, 147 (1932); *ibid.*, **246**, 6 (1937).

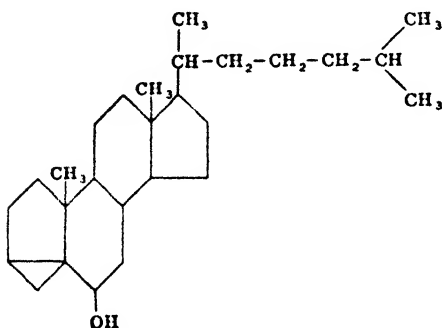
⁹⁶ Diels and Blumberg, *Ber.*, **44**, 2847 (1911); Bills and MacDonald, *J. Biol. Chem.*, **73**, 1 (1927).

⁹⁷ Wagner-Jauregg and Werner, *Z. physiol. Chem.*, **213**, 119 (1932).

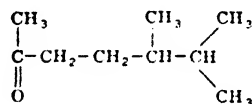
* In this compound "allo" does not indicate that the relationship of rings A/B is *trans* and therefore the prefix is not italicized.

cholesterol and dicholesteryl ether result from the *d*-ether, and with halogen acids the normal cholesteryl halides are obtained.⁹⁸ The behavior of the two ethers toward bromine is different. The normal *l*-ether gives a 5,6-dibromo addition product; the *d*-ether yields a 3,5,6-tribromo substitution-addition product.*

Similarly, acetylation of cholesteryl *p*-toluenesulfonate gives two products.⁹⁹ In the presence of potassium acetate a so-called *i*-cholesteryl acetate is produced which differs from the normal cholesteryl acetate, formed in the absence of potassium acetate, in that it has a very unreactive double bond or lacks a double bond completely. On hydrolysis, *i*-cholesterol, which is strongly dextrorotatory, is produced. The tentative formulation, II, that has been offered for *i*-cholesterol, indicates that an unusual kind of rearrangement is necessary to explain its formation.†



II *i*-Cholesterol
(provisional)



III Thujaketone

* Beynon, Heilbron, and Spring, *J. Chem. Soc.*, 907 (1936).

† In recent publications, Beynon, Heilbron, and Spring, *J. Chem. Soc.*, 406, 1459 (1937), have studied the two ethers further. In addition to the reactions given above, they are distinguished by their behavior when heated with nitric acid. The *l*-ether gives 6-nitrocholesteryl methyl ether, and the *d*-ether, 6-nitrocholesteryl nitrate. When the *d*-methyl ether is hydrogenated with platinum as a catalyst, cholestane is obtained in quantitative yield. Although this may be taken to indicate the presence of a double bond at C₆:C₄, this conclusion seems unlikely for the *d*-ether is not acted upon by ozone and does not give a coloration with tetranitromethane. Likewise a double bond at C₄:C₅ (allocholesterol structure) is improbable, since the *d*-ether does not give a positive Rosenheim reaction (p. 1272). The sign of the rotation suggests that the *d*-ether is probably derived from *i*-cholesterol, and this conclusion is in agreement with the above reactions.

⁹⁹ Wallis, Fernholz, and Gephart, *J. Am. Chem. Soc.*, **59**, 137 (1937).

† The formulation (II) of *i*-cholesterol has been questioned by Beynon, Heilbron, and Spring, *J. Chem. Soc.*, 1459 (1937), but has been accepted by Butenandt and Grosse, *Ber.*, **70**, 1446 (1937). The first group of workers have shown that *i*-cholesterol may be obtained directly from cholesterol *p*-toluenesulfonate by hydrolyzing in aqueous acetone in the presence of potassium acetate. Butenandt has prepared the methyl ethers of *i*-pregnenolone and of *i*-androstenediol, and thus it is apparent that *i*-derivatives may be obtained from any of the sterols containing a C₃-OH and a C₅:C₆ ethylenic bond.

The C₁₇ Side Chain. Except in the pregnanediols, the C₁₇ side chain of the sterols is an isoöctyl or substituted isoöctyl group. All the sterols with unsaturated side chains have the ethylene bond at C₂₂. This is doubtless of biogenetic significance. In determining the structure of such a sterol, the double bond of the side chain is placed by treating with ozone, which acts on the ethylene linkage to give a volatile aldehyde. Thus, ethylisopropylacetaldehyde is obtained from stigmasterol, and methylisopropylacetaldehyde from ergosterol and its irradiation products.¹⁰⁰

Chromic acid oxidation of the acetylated saturated sterols, or of the hydrocarbons from the sterols, cleaves the side chain at the linkages with tertiary carbon atoms, C₁₇—C₂₀, and C₂₄—C₂₅ or C₂₃—C₂₄.^{*} The yields are low, but the products are important both theoretically and practically. By cleavage of the C₁₇—C₂₀ bond volatile and non-volatile ketones are formed.¹⁰¹ The volatile ketones aid in determining the length and structure of the side chain. For example, from cholesterol (dihydrocholesterol), isohexyl methyl ketone¹⁰² has been isolated, and from α -ergosterol, a partially hydrogenated ergosterol, the optically active thujaketone (III).⁹³ The non-volatile ketones, the hydroxyetiocholanones or etioallocholanones, are used in the production of male sex hormones (p. 1371).

Oxidative cleavage at C₂₄—C₂₅ or C₂₃—C₂₄ forms volatile ketones and bile acid derivatives. The latter are of great importance in determining the stereochemical relationship of the nucleus and the C₃—OH group. As an illustration, acetyldihydrocholesterol may be considered. The bile acid isolated from its oxidation mixture proved to be β -3-hydroxyallocholanic, and this served as one way of establishing the spatial relation of the hydroxyl and of rings A, B.¹⁰³

The Nuclear Unsaturation. Nearly all the natural sterols are

¹⁰⁰ Reindel and Kipphan, *Ann.*, **493**, 181 (1932); Guiteras, Nakamiya, and Inhoffen, *Ann.*, **494**, 116 (1932).

^{*} The products formed by cleavage at the tertiary carbon linkages at C₁₄—C₁₇ and C₂₀—C₂₁ have also been reported. From the oxidation products of dibromocholesteryl acetate, Kuwada [*J. Pharm. Soc. Japan*, **56**, 75 (1936); *C.A.*, **31**, 2224 (1937)] has isolated a dibromo acid, which, on debromination with zinc, gave 3-hydroxy- Δ^4 -etiobilanic acid, m.p. 251°. This was subsequently [*J. Pharm. Soc. Japan*, **56**, 631 (1936); *C.A.*, **31**, 2224 (1937)] identified by conversion to etiobilanic acid (p. 1244). A small amount of pregnen-3-ol-20-one acetate, m.p. 186, the product formed by oxidation of dihydrocholesteryl acetate at C₂₀, has been isolated by Fujii and Matsukawa, *J. Pharm. Soc. Japan*, **56**, 138 (1936).

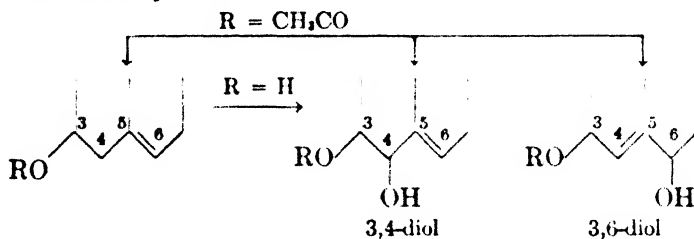
¹⁰¹ Ruzicka, Goldberg, and Brüngger, *Helv. Chim. Acta*, **17**, 1389 (1934).

¹⁰² Windaus and Resau, *Ber.*, **46**, 1246 (1913); Windaus and Neukirchen, *Ber.*, **53**, 1915 (1919); Windaus, *Z. physiol. Chem.*, **145**, 177 (1925). Cf. Ruzicka, *et al.*, reference 101.

¹⁰³ Fernholz and Chakravorty, *Ber.*, **67**, 2021 (1934).

unsaturated in the 5,6-position. The method of determining the position of this double bond has been illustrated in the discussion of cholesterol (p. 1236), and has been tested on a sufficiently large number of sterols to establish its general applicability. The double bond in the 5,6-position is more reactive, apparently, than a double bond placed elsewhere in the molecule, including the side chain. The addition of hydrogen to this double bond in neutral solvents takes place in such a way that the added hydrogen at C₅ is probably *trans* to the C₁₀—CH₃. Bromine, on the other hand, adds to give a *cis* compound, since the ketone formed by oxidizing the dibromide brominates at C₄ rather than at C₂.¹⁰⁴

The methylene groups adjacent to an ethylenic bond at C₅:C₆ are easily oxidized to carbinol or ketones. If the mild oxidizing agent selenium dioxide¹⁰⁵ is used in suitable solvents (acetic acid, etc., but not alcohol), cholesterol yields $\Delta^{5,6}$ -cholestene-3,4-diol, m.p. 176°, [α]_D-60. With cholesteryl acetate nearly equal amounts of the 3,4-diol and of Δ^4 -cholesten-3,6-diol, m.p. 257°, [α]_D+6, are formed. The reaction is shown schematically below:



At first the 3,6-diol was incorrectly formulated, but the structures of both compounds are now well established, largely through the work of Butenandt. The positions of the hydroxyl groups in the 3,4-diol are shown by the fact that gentle oxidation converts the compound through the stage of a dialdehyde (opening of ring A) to the known dihydro-Diels' acid (p. 1275). The structure of the 3,6-diol is most simply established through dehydrogenation by Oppenauer's method (note, p. 1239) to cholestan-3,6-dione (p. 1274).

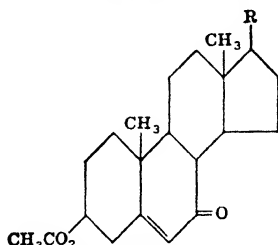
With chromic acid and potassium permanganate as oxidizing agents the action is principally on carbon 7.¹⁰⁶ Chromic acid at 40° converts

¹⁰⁴ Butenandt and Schramm, *Ber.*, **69**, 2289 (1936).

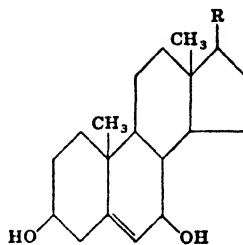
¹⁰⁵ Rosenheim and Starling, *J. Chem. Soc.*, 377 (1937); Butenandt and Hausmann, *Ber.*, **70**, 1154 (1937).

¹⁰⁶ Mauthner and Suida, *Monatsh.*, **17**, 496 (1896); Windaus, *Ber.*, **53**, 488 (1920); Windaus, Lettré, and Schenck, *Ann.*, **520**, 98 (1935); Dimroth and Trautmann, *Ber.*, **69**, 609 (1936); Wunderlich, *Z. physiol. Chem.*, **241**, 116 (1936); Linsert, *ibid.*, **241**, 125 (1936); Barr, Heilbron, Parry, and Spring, *J. Chem. Soc.*, 1437 (1936); Marker, Kamm, Fleming, Popkin, and Wittle, *J. Am. Chem. Soc.*, **59**, 619 (1937).

sterol esters to 7-ketosterols (IV) which, on reduction with aluminum isopropoxide, give mixtures of isomeric 7-hydroxy sterols (V) in which the lower-melting α -isomer predominates. The β -stereoisomer (β -7-hydroxy) may be produced directly by permanganate oxidation of the acid phthalate ester of the sterol. By benzoylation and pyrolysis the newly introduced hydroxyl groups are split out to give dehydrosterols with the conjugated system $C_5 : C_6 \cdot C_7 : C_8$.



IV 7-Ketosterol acetate



V 7-Hydroxysterol

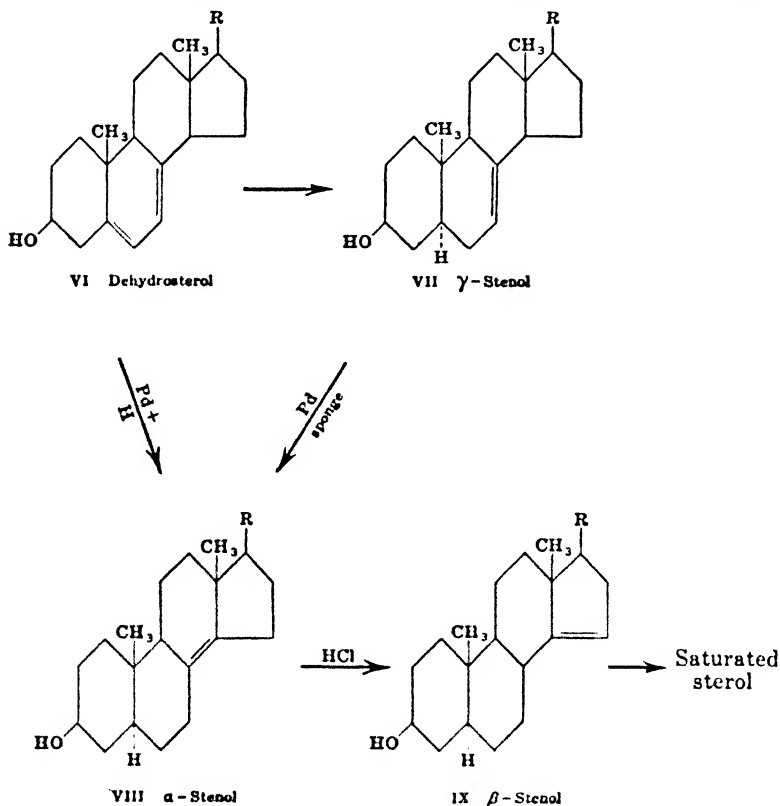
When a dehydrosterol (VI) is reduced with sodium and alcohol, only one of the double bonds is saturated. The resulting γ -stenol (VII), in which the double bond is probably at $C_7 : C_8$, is rearranged into an α -stenol (VIII) by shaking with palladium sponge. The double bond of the α -stenol is resistant to catalytic hydrogenation, and is probably located at $C_8 : C_{14}$. The α -stenol may be produced directly by catalytic hydrogenation of the dehydrosterol in the presence of palladium; only one mole of hydrogen is taken up. Although the α -stenol cannot be hydrogenated, it can be rearranged by treatment with hydrogen chloride in chloroform solution to a β -stenol (IX) which takes up hydrogen readily to give the completely saturated sterol.¹⁰⁷

With oxygen the dehydrosterols form peroxides when irradiated in alcohol solution in the presence of a sensitizing dye like eosin. These peroxides are unique among organic peroxides in that they are stable toward alkaline reagents; for example, their acetates may be saponified with dilute alkali without decomposition. Since 1,2-addition of oxygen to the 5,6-ethylenic bond would hardly account for this stability, 1,4-addition to the conjugated system (p. 575) must be considered also. The direct evidence¹⁰⁸ from chemical transformations does not differentiate well between these two possibilities. Irradiation with visible light in the absence of oxygen but with a sensitizer leads to the production of pinacols. These are formed with the loss of one molecule of water from

¹⁰⁷ Achtermann, *Z. physiol. Chem.*, **225**, 141 (1934); Laucht, *ibid.*, **237**, 236 (1935); Dimroth and Trautmann, *Ber.*, **69**, 669 (1936).

¹⁰⁸ Windaus and Brunken, *Ann.*, **460**, 225 (1928); Achtermann, *Z. physiol. Chem.*, **217**, 281 (1933); Müller, *ibid.*, **231**, 75 (1935); Fieser, p. 174.

two molecules of the dehydrosterol. They are sparingly soluble, unstable compounds, and when heated above their melting points, or when boiled in acetic anhydride, the pinacols lose methane and are converted into "norsterols," in which the $C_{10}-CH_3$ is missing.¹⁰⁷ An example of



such a transformation is cited later in connection with the proof of the structure of ergosterol.

The dehydrosterols have a characteristic absorption spectrum in the ultra-violet within the range 260–300 $m\mu$, with maxima at *ca.* 270 $m\mu$, 280 $m\mu$, and 295 $m\mu$.^{*} The absorption is due entirely to the conjugated system in ring B, and the absolute position of the bands is unaffected by the surrounding structure.¹⁰⁹ The intensity of the absorption, however, varies as the surrounding structure is modified.

The Color Reactions of the Sterols. The sterols give a number of color reactions which are useful for preliminary identification and

^{*} For typical curve (ergosterol) see Morton, "The Application of Absorption Spectra to the Study of Vitamins and Hormones," Hilger, London (1935), p. 8.

¹⁰⁹ Dimroth and Trautmann, *Ber.*, **69**, 669 (1936).

quantitative determination, but are not specific for the sterols. The most important follow:

The Salkowski Reaction.¹¹⁰ A chloroform solution of a sterol is shaken with an equal volume of concentrated sulfuric acid. After separation of the layers, a red color is generally present in the chloroform layer and a green fluorescence in the sulfuric acid. The mycosterols and some of the plant sterols give a reverse Salkowski reaction, in that the sulfuric acid layer becomes red and the chloroform remains colorless.

The Liebermann-Burchard Reaction.^{111,112} A chloroform solution of a sterol is treated with a few drops of acetic anhydride and concentrated sulfuric acid, the temperature being maintained at 15–20°. A color soon develops in the solution, passing from rose-red through blue to green. The time of color development is characteristic of the specific sterol, and in general the saturated sterols give this color sequence much more slowly than the unsaturated.¹¹³ If the solid sterol is used instead of a chloroform solution, the test is known as the Liebermann Reaction.

The Rosenheim Test.¹¹⁴ Either a chloroform solution of the sterol or the free sterol is treated with 90 per cent chloroacetic acid. Sterols or sterol derivatives containing or potentially containing a conjugated system of carbon atoms¹¹⁵ gives an intense red color initially, which in some instances changes in a short time to bright blue.

Since these tests are carried out in the presence of dehydrating agents, the course of the reaction may involve dehydration as a first stage. The colored substances are probably halochromic salts.

Molecular Compounds. One of the characteristics of the sterols is their ability to form molecular compounds and mixed crystals with other sterols. By means of melting-point diagrams, Lettré¹¹⁶ has studied the relationship between these tendencies and structure. If cholestanol and coprostanol (coprosterol) and their epimers are used as examples representing all the possible arrangements with respect to the C₁₀—CH₃ group at the points of asymmetry, C₃ and C₅, the following relationships obtain:

	C ₃ —OH	C ₅ —H		C ₃ —OH	C ₅ —H
Cholestanol	<i>cis</i>	<i>trans</i>	Coprostanol	<i>cis</i>	<i>cis</i>
Epicholestanol	<i>trans</i>	<i>trans</i>	Episcoprostanol	<i>trans</i>	<i>cis</i>

¹¹⁰ Salkowski, *Z. physiol. Chem.*, **57**, 523 (1908).

¹¹¹ Liebermann, *Ber.*, **18**, 1803 (1885).

¹¹² Burchard, *Chem. Zentr.*, **61**, [I], 25 (1890).

¹¹³ Schoenheimer and Dam, *Z. physiol. Chem.*, **215**, 59 (1933); Morgareidge, *J. Biol. Chem.*, **109** (Proceedings), lxvii (1935).

¹¹⁴ Rosenheim, *Biochem. J.*, **23**, 47 (1929); Rosenheim and Callow, *ibid.*, **25**, 74 (1931).

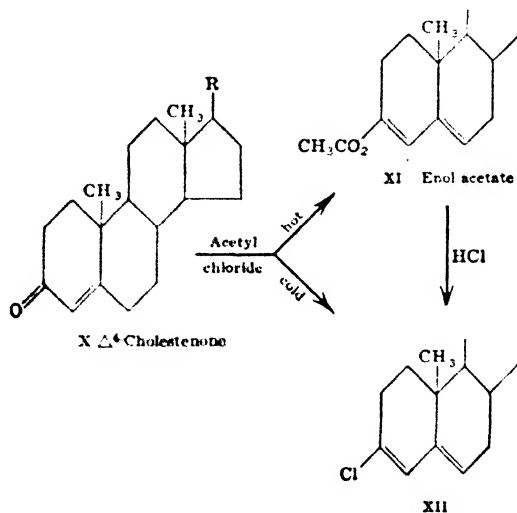
¹¹⁵ Schoenheimer and Evans, *J. Biol. Chem.*, **114**, 567 (1936).

¹¹⁶ Lettré, *Ann.*, **495**, 41 (1932).

Compound formation takes place between cholestanol and *epicoprostanol*, or between *coprostanol* and *epicholestanol*. Molecular compounds are formed, therefore, by pairs which are structurally dissimilar with respect to both the C_3-OH and the C_5-H . The spatial position of the hydroxyl group appears to be the determining factor, since masking the hydroxyl by acetylation destroys the capacity for compound formation. One of the components of a pair may be replaced by a structurally similar compound; for example, ergostanol, 3(*cis*)-hydroxy-24-methylcholestane, forms a molecular compound with *epicoprostanol*.

Mixed crystals are formed by sterols and 5,6-dihydrosterols, as with the pairs cholesterol-cholestanol and ergosterol-dihydroergosterol. Here, too, the hydroxyl group plays a part, but a minor one, for acetylation does not interfere with mixed crystal formation, whereas replacement of the hydroxyl by hydrogen does.

Bromination of Sterol Ketones. Substitution of bromine in the sterol ketones probably takes place by addition of the halogen to the



double bond of the enolic form of the ketone followed by loss of hydrogen bromide from the addition product. Support for such a mechanism comes from the behavior of the sterol ketones toward acid chlorides: At room temperature unsaturated chlorides are formed, but in boiling toluene, where hydrogen chloride is eliminated as rapidly as it is produced, enol esters are obtained.¹¹⁷ Thus at lower temperatures Δ^4 -cholestenone (X) is converted by acetyl chloride into 3-chloro-3,5-cholestadiene (XII), and at higher temperatures the enol acetate (XI) is

¹¹⁷ Ruzicka and Fischer, *Helv. Chim. Acta*, **19**, 806 (1936).

formed. Probably the enol acetate results at lower temperatures, too, but is acted upon by the liberated hydrogen chloride.

The monobromo ketones, 2-bromocholestanone (XIII) and 4-bromocoprostanone, from cholestanone and coprostanone, respectively, lose hydrogen bromide when treated with pyridine. In both, an ethylenic linkage in conjugation with the carbonyl double bond is produced. Since the yields with 2-bromocholestanone are smaller than with 4-bromocoprostanone, it is reasonable to assume that the bromine is held more firmly at C₂ than at C₄.¹¹⁸ Both these monobromo ketones form dibromo derivatives. Opinions differ as to whether the product from 2-bromocholestanone is 2,2-dibromo (Ruzicka) or 2,4-dibromo (XIV) (Butenandt), but there is agreement that 4,4-dibromocoprostanone is the probable structure of the isomer.¹¹⁹ Ruzicka¹²⁰ bases his formulation on the fact that dibromocholestanone reacts with *o*-phenylenediamine to give a quinoxaline. Butenandt,¹¹⁹ on the other hand, finds that potassium acetate converts dibromocholestanone to cholestan-3,4-dione (XV).^{*} The structure of this dione follows from its conversion by hydrogen peroxide to dihydro-Diels' acid (XVI), and its preparation from cholesterol hydrochloride (XVII).

Hydrogen chloride adds to the double bond of cholesterol to give 3-hydroxy-5-chlorocoprostanone (XVII) (probable structure in analogy to addition of bromine, p. 1269). After oxidation to the corresponding ketone (XVIII), bromine substitutes at C₄ with the formation of a 4-bromo-5-chloro ketone (XIX). Removal of hydrogen and chlorine is readily accomplished to produce an α, β -unsaturated monobromo ketone. Treatment of the bromochloro ketone with potassium acetate in acetic acid gives cholestan-3,4-dione and some cholestan-3,6-dione (XX); the formation of the latter probably takes place through allyl rearrangement.¹¹⁹ Cholestan-3,4-dione absorbs strongly at 280 m μ , and appears

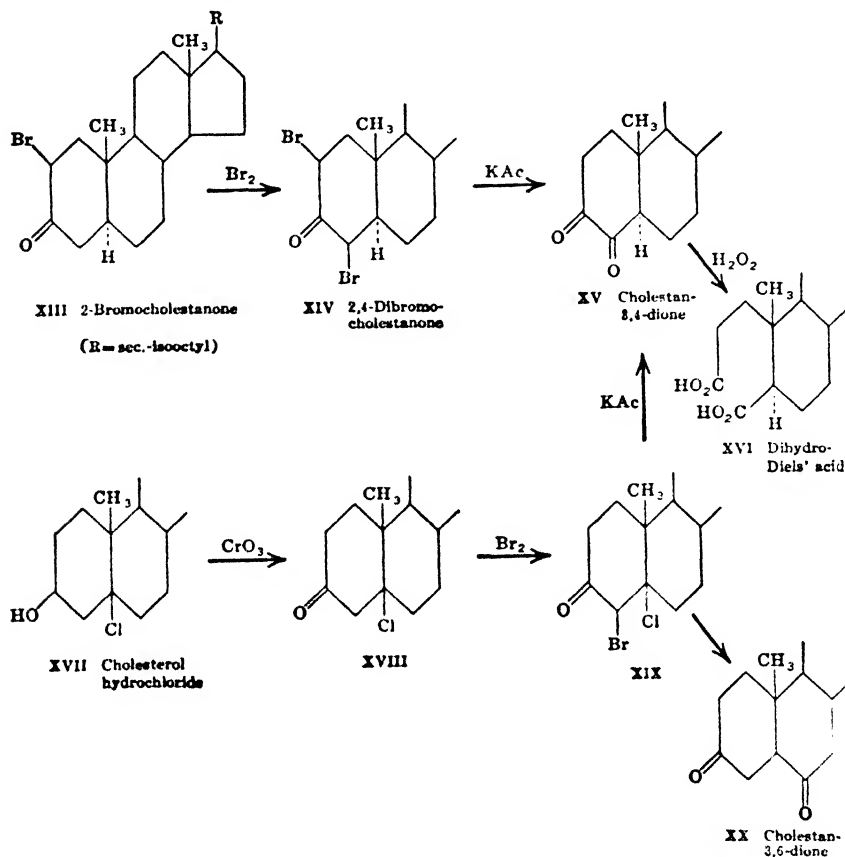
¹¹⁸ Butenandt and Wolff, *Ber.*, **68**, 2091 (1935).

¹¹⁹ Butenandt, Schramm, Wolff, and Kudsus, *Ber.*, **69**, 2779 (1936).

¹²⁰ Ruzicka, Bosshard, Fischer, and Wirz, *Helv. Chim. Acta*, **19**, 1147 (1936). Cf. Dane, Wang, and Schulte, *Z. physiol. Chem.*, **245**, 80 (1937).

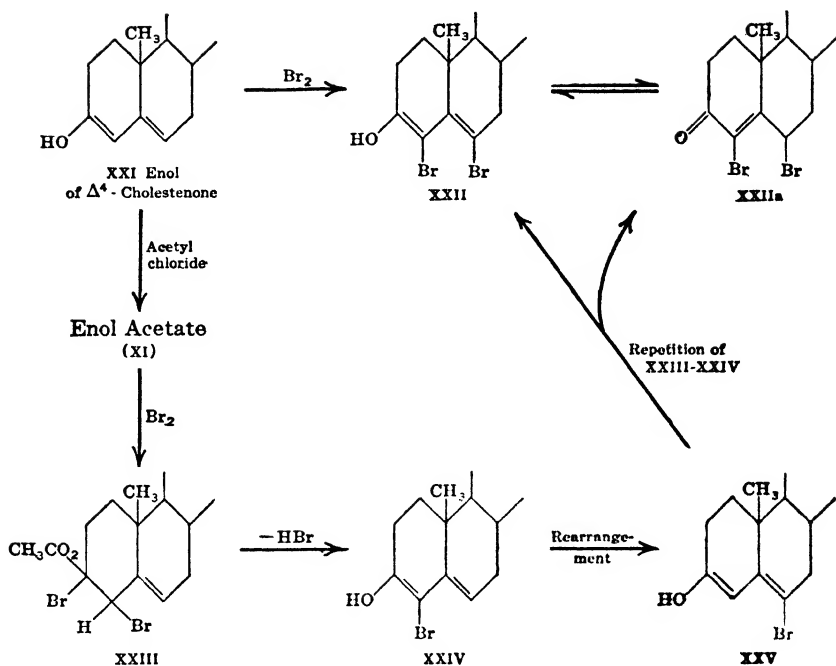
^{*} Later work by Inhoffen, *Ber.*, **70**, 1695 (1937), confirms Butenandt's formulation of the dibromo product from cholestanone as 2,4-dibromocholestanone. When the dibromo ketone is treated with potassium benzoate in butyl alcohol, two monobenzoate products are obtained. The less soluble of these monobenzoates, m. p. 177°, $[\alpha]_D + 25.9$, yields cholestan-3,4-dione on hydrolysis, but the more soluble benzoate, m. p. 137–138°, $[\alpha]_D + 58$, gives cholestan-2,3-dione. The structure of the 2,3-dione was established by oxidizing it to the corresponding dicarboxylic acid, a compound previously investigated by Windaus and Uibrig, *Ber.*, **47**, 2384 (1914). With *o*-phenylenediamine, cholestan-2,3-dione forms the same quinoxaline that Ruzicka obtained from the dibromocholestanone. As these results show, two kinds of reaction take place in the treatment with potassium benzoate—substitution of benzoyl for bromine and the production of an ethylenic bond. The enols formed on hydrolysis rearrange to give the two diones.

to have the structure of a diosphenol, since it gives a red color with ferric chloride, and is converted by acetylation into a mono-enol acetate with strong absorption at 240–250 $m\mu$ (*cf.* cholestenone, p. 1239).

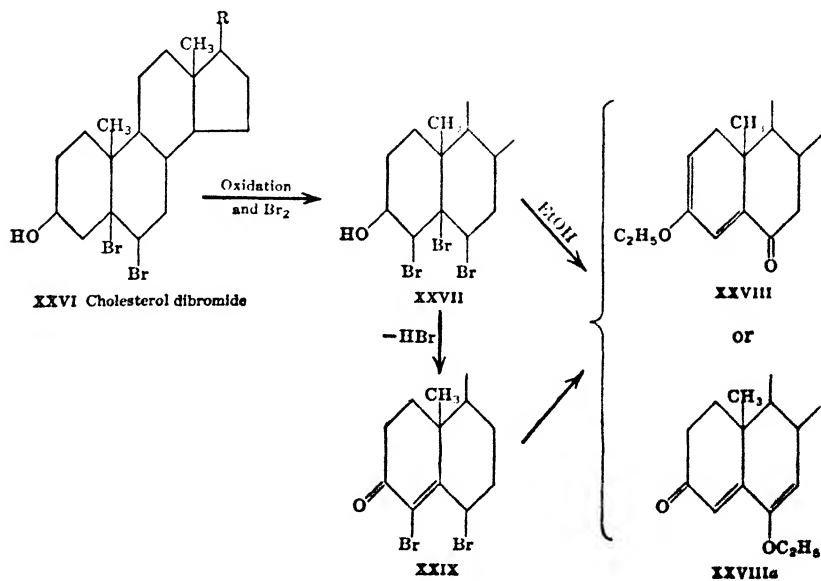


A more complex situation is presented in the interaction of bromine and Δ^4 -cholestenone (X). Bromination of Δ^4 -cholestenone in ether-acetic acid takes place at C_4 and C_6 . The entrance of bromine is conditioned by the presence of hydrogen bromide, since no bromination occurs in the presence of potassium acetate. The mechanism of the reaction is interaction between an enol and bromine. This is shown by the fact that the enol acetate of cholestenone takes up one molecule of bromine to give a monobromo unsaturated ketone. In the presence of hydrogen bromide, the latter is substituted to a dibromo ketone. The course of the transformation, XXI–XXV, is that of Inhoffen.¹²¹

¹²¹ Inhoffen, *Ber.*, **69**, 2141 (1936).



The dibromo ketone formed by oxidizing cholesterol dibromide (XXVI) reacts with bromine to give two stereoisomeric products.



Bromination in ether gives a bromo ketone dibromide¹²² (XXVII) of m.p. 137–138°; in acetic acid an isomer of m.p. 106° is formed.¹⁰⁴ Both products react with alcohol to give the same cholestendione ethyl ether (XXVIII or XXVIIIa). The tribromo ketone loses hydrogen bromide, when treated with potassium acetate, to form an unsaturated dibromo ketone (XXIX) which absorbs strongly at 248 m μ , and which goes over to the dione ethyl ether when heated with ethyl alcohol and hydrogen bromide. The dibromo ketone is probably an intermediary product in the conversion.

The Zoösterols

Cholesterol is the principal animal sterol, and is found in all animal tissues. At one time, it was thought that all the sterols associated with the animal kingdom had the same carbon content as cholesterol. One of the supporting facts for this view was the isolation in 1872 of "ischolesterol" from wool fat.¹²³ Since that time this "sterol" has been resolved into two compounds: agnosterol,¹²⁴ C₃₀H₄₈O; and lanosterol,¹²⁵ C₃₀H₅₀O. Apparently neither of these substances is a sterol.¹²⁶ The isolation from animal tissues and excreta of a number of sterols of higher and lower carbon content than cholesterol has definitely disposed of the older belief.

Cholesterol [3(cis)-Hydroxy-5-cholestene]. The cholesterol content of different animal tissues varies from a few hundredths of a per cent to 4-5 per cent.* Although cholesterol is doubtless of great importance to animal life, no specific function can be assigned to it. Generally it is described as an essential constituent of cells, but its synthesis and metabolism are obscure. Biochemical investigations have established the following facts:

1. Cholesterol, allocholesterol and ostreasterol appear to be the only sterols that are absorbed from the intestinal tract.¹²⁷ Of these only cholesterol is readily absorbed. The allocholesterol may rearrange to cholesterol in the intestine.¹²⁸

¹²² Inhoffen, *Ber.*, **69**, 1134, 1702 (1936).

¹²³ Schulze, *Ber.*, **5**, 1075 (1872); **6**, 251 (1873); **31**, 1200 (1898).

¹²⁴ Windaus and Tschesche, *Z. physiol. Chem.*, **190**, 51 (1930).

¹²⁵ Dorée and Garratt, *J. Soc. Chem. Ind.*, **52**, 141T, 355T, (1933).

¹²⁶ Schulze, *Z. physiol. Chem.*, **238**, 35 (1936).

* For cholesterol content of various tissue see Lettré and Inhoffen, *Monograph*, p. 98.

¹²⁷ Schoenheimer, *Science*, **74**, 579 (1931); *Dam. Biochem. J.*, **28**, 815, 820 (1934); Sperry and Bergmann, *J. Biol. Chem.*, **119**, 171 (1937).

¹²⁸ Schoenheimer, *Dam*, and v. Gottberg, *J. Biol. Chem.*, **110**, 667 (1935). Later work by Schoenheimer, reference 139, shows that the allocholesterol used was impure.

2. Vertebrates are capable of synthesizing cholesterol. A mouse on a cholesterol-free diet can synthesize 1-2 mg. per day; the rate of synthesis is dependent on the supply of cholesterol from external sources, the synthetic activity serving to make up any deficit.¹²⁹

3. Destruction of cholesterol occurs in the organism, but the intermediary metabolism is unknown. Some of the sterol is excreted in the bile, and dihydrocholesterol is excreted through the wall of the large intestine.* Cholesterol is excreted in the urine to the extent of 1 mg. per liter.¹³⁰

4. About one-third of the cholesterol of the blood is present as esters of the higher fatty acids, but in certain diseases the ratio of cholesterol to cholesterol ester is reversed in favor of the ester.*

Epicholesterol [3(*trans*)-Hydroxy-4-cholestene]. The stereoisomer of cholesterol, *epicholesterol*, has not been found in nature, but is obtained by synthetic methods.¹³¹ By the procedure of Marker cholesteryl-magnesium chloride is treated with oxygen and the oxygenated product hydrolyzed to a mixture of cholesterol and *epicholesterol*. Ruzicka reduces Δ^5 -cholestenone (see below) catalytically in the presence of the Raney-nickel catalyst to a mixture of the two sterols. Both workers have separated the mixture by precipitating the cholesterol with digitonin and extracting the *epicholesterol* which does not form an insoluble digitonide. On a large scale *epicholesterol* can be separated from cholesterol by crystallizing first the acetates and then the benzoates from ethyl alcohol.

By the use of cholesteryl chloride (p. 1260), a compound in which inversion at C₃ has already been produced, Marker¹³¹ in his latest work has developed another method for the conversion of cholesterol to *epicholesterol*. Oxidation of the chloride with chromic acid and acetic acid at 55° gives 7-ketocholesteryl chloride in a yield of 25 per cent, and from the ketonic chloride a mixture of 7-ketocholesteryline and 7-keto-*epicholesterol* is formed by treatment with potassium acetate. *Epi*-cholesterol is obtained when 7-keto-*epicholesterol* is reduced by the method of Wolff-Kishner.

The Cholestenones. When cholesterol dibromide is oxidized with chromic acid and debrominated with zinc and acetic acid, Δ^4 -cholestenone, m.p. 80°, $[\alpha]_D + 88.8$, is obtained (p. 1239). If the debromination

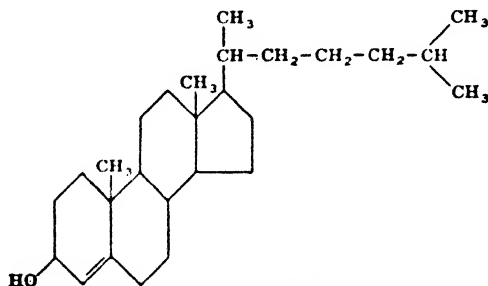
¹²⁹ Schoenheimer and Breusch, *J. Biol. Chem.*, **103**, 439 (1933).

¹³⁰ Butenandt and Dannenbaum, *Z. physiol. Chem.*, **248**, 151 (1937).

* For discussion of cholesterol metabolism see Bills, *Physiol. Rev.*, **15**, 1 (1935) and current volumes of "Ann. Rev. of Biochem."

¹³¹ Marker, Oakwood, and Crooks, *J. Am. Chem. Soc.*, **58**, 481 (1936); Marker, Kamm, Oakwood, and Laucius, *ibid.*, **58**, 1948 (1936); Ruzicka and Goldberg, *Helv. Chim. Acta*, **19**, 1407 (1936).

is carried out in weakly acidic alcohol, a double bond is introduced without rearrangement, and Δ^5 -cholestenone, m.p. 127° , $[\alpha]_D - 4.2$,¹³² is obtained. The latter compound is readily rearranged into the former. An important place in the metabolism of cholesterol has been suggested for Δ^4 -cholestenone. The sterol mixture present in the feces of man and other Carnivora is largely coprosterol with small amounts of dihydrocholesterol and traces of cholesterol.¹³³ Since the bile contains cholesterol and some dihydrocholesterol,¹³⁴ there must be a biochemical mechanism to account for the formation of the stereoisomer coprosterol [3(*cis*)-hydroxycoprostan-]. At one time allocholesterol (XXX) was thought to be the intermediary substance, but careful search has failed



XXX Allocholesterol

to reveal even traces of allocholesterol in animal tissues.¹³⁵ Schoenheimer¹³⁶ has suggested that Δ^4 -cholestenone is the intermediary substance in the formation of coprosterol. As evidence of this, added Δ^4 -cholestenone brings about an increase in fecal coprosterol in dogs on a meat diet but not in those on a dog biscuit diet; with the latter diet, the cholesterol output is increased. Similarly, the conversion of coprostanone to coprosterol has been shown with the use of the deuterium derivative, coprostanone-4,5- d_2 . The colon appears to be the site of formation of coprosterol.¹³⁷

Allocholesterol. Interest in allocholesterol as an intermediary substance in the metabolism of cholesterol is of many years' duration; yet pure allocholesterol was first prepared in 1936. The difficulty presented in the preparation of this substance well illustrates the nature of

¹³² Butenandt and Schmidt-Thomé, *Ber.*, **69**, 882 (1936).

¹³³ Schoenheimer and co-workers, *Z. physiol. Chem.*, **192**, 73 (1930).

¹³⁴ Schoenheimer and Hrdina, *ibid.*, **212**, 161 (1932).

¹³⁵ Schoenheimer, Dam, and Gottberg, *J. Biol. Chem.*, **110**, 659, 667 (1935); Evans, *ibid.*, **115**, 449 (1936).

¹³⁶ Schoenheimer, Rittenberg, and Graff, *ibid.*, **111**, 183 (1935). See, also, Rosenheim and Webster, *Nature*, **126**, 474 (1935).

¹³⁷ Gardner, Gainsborough, and Murray, *Biochem. J.*, **29**, 1139 (1935).

sterol chemistry. Cholesterol adds hydrogen chloride in anhydrous media to form the so-called cholesterol hydrochloride (XVII). When the hydrochloride is treated with anhydrous potassium acetate, hydrogen and chlorine are removed, and a new substance (the old allocholesterol), m.p. 120–121°, isomeric with cholesterol, is obtained. When reduced, this substance gives a poor yield of coprosterol and some cholesterol—presumably the low yield was due to a rearrangement in part back to cholesterol.¹³⁸ Actually the product isolated is a mixture of allocholesterol and cholesterol. This has been shown by Evans and Schoenheimer,¹³⁹ who reduced Δ^4 -cholestenone with aluminum isopropoxide to form a molecular addition compound, m.p. 141°, of allocholesterol and *epiallocholesterol*. Resolution of the molecular compound could not be effected by crystallization, but digitonin precipitation removed the allocholesterol. Only coprosterol could be isolated from the product of catalytic hydrogenation of allocholesterol, but from *epiallocholesterol* both *epicoprosterol* and *epidihydrocholesterol* were obtained. Both allocholesterols are easily dehydrated by alcoholic hydrochloric acid. The product of the dehydration is 3,5-cholestadiene, although it was first reported to be 2,4-cholestadiene.

When Δ^4 -cholestenone is reduced with sodium, a molecular compound, m.p. 160°, of dihydrocholesterol and *epiallocholesterol* is obtained.¹⁴⁰ The molecular compound was at one time regarded as an isomer of cholesterol and was known as β -cholesterol.

The Cholestadienes. Cholesterol can be dehydrated by distilling from anhydrous copper sulphate or by various other methods. The dehydration product is known as cholesterolene. Recently Bergmann¹⁴¹ has studied the structure of this compound and from its reactions has concluded that it is a 3,5-cholestadiene. As proof of the correctness of this formulation 3,5-cholestadiene was prepared from 7-ketocholesteryl acetate by splitting off acetic acid and reducing the carbonyl at C₇ by the Wolff-Kishner method. In this conversion the carbonyl at C₇ served to stabilize the double bond at C₅. An isomer of cholesterolene, 2,4-cholestadiene, may be obtained by heating cholesterol over aluminum oxide and distilling the product. On treatment with hydrochloric acid, 2,4-cholestadiene is rearranged to the 3,5-diene.

The two cholestadienes show a number of interesting differences.

¹³⁸ Windaus, *Ann.*, **447**, 233 (1926); **453**, 101 (1927).

¹³⁹ Evans and Schoenheimer, *J. Am. Chem. Soc.*, **58**, 182 (1936); *J. Biol. Chem.*, **114**, 567 (1936).

¹⁴⁰ Diels and Linn, *Ber.*, **41**, 260 (1908); Evans, Jr. and Schoenheimer, *J. Biol. Chem.*, **115**, 17 (1936); cf. reference 97.

¹⁴¹ Stavely and Bergmann, *J. Org. Chem.*, **1**, 567, 575 (1936). A good bibliography to the earlier work is given in the first of these articles.

The physical properties are: 3,5-cholestadiene, m.p. 78–79°, $[\alpha]_D -64$ (CHCl_3); 2,4-cholestadiene, m.p. 63°, $[\alpha]_D +114$ (CHCl_3). The shift in specific rotation from negative in the 3,5-diene to positive in the 2,4-diene is further evidence that the assigned structures are correct, since Callow and Young (p. 1261) have noted that compounds with unsaturation at Δ^5 show a less positive rotation than those with unsaturation at Δ^4 . The 3,5-diene adds maleic anhydride (p. 593) with difficulty and the acid formed by hydrolysis gives insoluble alkaline salts, thus differing from the other known maleic acid addition compounds in this group. On the other hand, the maleic acid addition product of 2,4-cholestadiene forms soluble alkaline salts. 3,5-Cholestadiene has an absorption spectrum with maxima at 229, 235 and 244 $m\mu$, while that of the 2,4-diene is at 260 $m\mu$. On catalytic reduction 3,5-cholestadiene is hydrogenated to cholestane (80 per cent) and coprostane (20 per cent), but 2,4-cholestadiene is quantitatively converted into coprostane.

The problem of the structure of cholesterolene is not completely solved, however, for there is a modification of this compound with a specific rotation of -97.5 . The course of the addition of maleic acid anhydride to 3,5-cholestadiene also requires study, since it is unusual for a compound with unsaturation in two different rings to react with maleic anhydride.

Sterols from Lower Forms of Animal Life. From oysters and clams the characteristic sterol, oystersterol, $\text{C}_{29}\text{H}_{48}\text{O}$, has been isolated.¹⁴² Its structure is not completely known, but hydrogenation converts it to sitostanol (γ -sitostanol?). An oystersterol II, m.p. 121–122°, has been described also.

The eggs and oil of the silkworm contain a mixture of cholesterol and sitosterol which at one time was regarded as a definite compound.¹⁴³ It is uncertain whether the sitosterol originates from the diet or whether it is synthesized by the worm. These and other sterols from the lower forms of animal and plant life* are of interest, as they suggest a relationship between the stage of evolution and the type of sterol formed. Until the role of the sterols is understood, however, it is impossible to evaluate such an evolutionary process if one exists.

Pregnanediol and Allopregnanediol. From pregnancy urine of women two isomeric dihydroxysterols, pregnanediol¹⁴⁴ and allopreg-

¹⁴² Bergmann, *J. Biol. Chem.*, **104**, 317, 553 (1934).

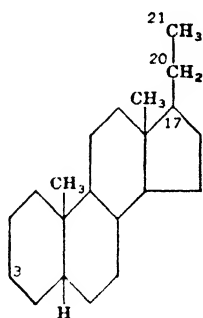
¹⁴³ Bergmann, *ibid.*, **107**, 527 (1934); **117**, 175 (1937).

* (Cf. list in monograph of Lettré and Inhoffen, p. 109; Schulze, reference 126.

¹⁴⁴ Butenandt, *Ber.*, **63**, 659 (1930); Butenandt, Hildebrandt, and Brücher, *Ber.*, **64**, 2529 (1931); Venning and Browne, *Proc. Soc. Exptl. Biol. Med.*, **34**, 792 (1936); Odell and Marrian, *Biochem. J.*, **30**, 1533 (1936).

nanediol,¹⁴⁵ $C_{21}H_{36}O_2$, have been isolated. Both compounds are presumably formed by *in vivo* reduction of the hormone progesterone (p. 1367). Pregnanediol is excreted as a glucuronate, from which it is split by boiling with hydrochloric acid. In the ninth month of pregnancy the amount of pregnanediol excreted amounts to 20–25 mg. per liter of urine. *Allo*pregnanediol is present in about one-fifth of this amount.

The structure of pregnanediol [3(*trans*), 20-dihydroxypregnane] has been established through its conversion *via* pregnanedione to the hydrocarbon pregnane (XXXI). The constitution of this hydrocarbon follows from the fact that it is formed by Clemmensen reduction of



XXXI Pregane
(17-ethyletiocholane)

etiocheryl methyl ketone (p. 1243). The allocation of the hydroxyl groups is most simply shown by the conversion of pregnanediol to progesterone (see p. 1369.) Similar transformations have established the structure of *allo*pregnanediol. Neither pregnanediol nor *allo*pregnanediol forms an insoluble digtonide and therefore the C_3-OH in both compounds is *trans* to the $C_{10}-CH_3$. When the mixture of pregnanediol and *allo*pregnanediol from pregnancy urine is oxidized with chromic acid, a mixture of the corresponding diketones is obtained.¹⁴⁶ Crystallization from acetone yields the more sparingly soluble *allo*pregnanedione, m.p. 199–200°. On catalytic reduction in acetic acid with platinum oxide *allo*pregnanedione is converted to an *allo*pregnanediol, m.p. 195–196°, which forms an insoluble digtonide. Thus the natural pregnanediols are compounds in which the C_3-OH has an “*epi*” configuration and the *allo*pregnanediol prepared by synthetic methods is one in which the C_3-OH has a “normal” configuration.

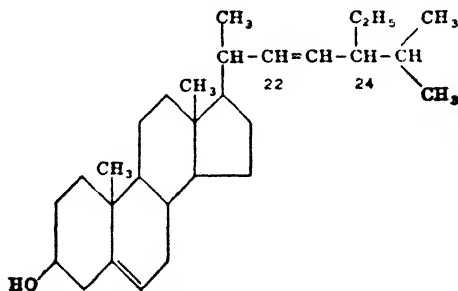
¹⁴⁵ Hartmann and Locher, *Helv. Chim. Acta*, **18**, 160 (1935).

¹⁴⁶ Marker, Kamm, Jones, and Oakwood, *J. Am. Chem. Soc.*, **59**, 614 (1937); Marker, personal communication.

The Phytosterols

A large number of sterols have been isolated from plants,* but most of them have been so poorly characterized that there is considerable uncertainty as to which are definite chemical individuals. Stigmasterol, $C_{29}H_{48}O$, is the only plant sterol to which a definite structure can be assigned. Sitosterol, which has been described as the chief plant sterol, appears to be a mixture of at least four components. To the specific plant sterols, α -spinasterol, $C_{28}H_{46}O$ (?), from spinach and senega root, fucosterol, $C_{29}H_{48}O$, from bladder wrack, and cinchol, $C_{29}H_{50}O$, from cinchona bark, definite structures cannot be assigned.

Stigmasterol [3(*cis*)-Hydroxy-24-ethyl-5,22-cholestadiene]. Although the plant sterols are widely distributed, only soy and calabar bean oils contain enough stigmasterol (XXXII) to be considered as sources of the sterol. From soy bean oil, stigmasterol is conveniently separated as its sparingly soluble acetate tetrabromide from the accom-



XXXII Stigmasterol

panying sitosterol. The structure of stigmasterol was determined by Fernholz,¹⁴⁷ building on the earlier work of Guiteras.¹⁴⁸ The steps in the structural investigation were the following: Determination of the empirical formula;^{92,93} characterization of part of the side chain and location of the side chain double bond by ozone degradation to ethylisopropylacetaldehyde;¹⁴⁸ oxidative degradation of the acetylated 5,6-dibromosterol, followed by debromination to β -3-hydroxy-5-bisnorcholenic acid; chromic acid oxidation of stigmasterol to β -3-hydroxy-norcholenic acid; and location of the nuclear double bond at C₅ : C₆ by a series of transformations analogous to those described for cholesterol (p. 1236 *et seq.*). Stigmasterol gives an insoluble diglucoside. This sterol

* For partial lists see "Biochemisches Handlexikon" (1923), X, 179; (1933), XIV, 826.

¹⁴⁷ Fernholz, *Ann.*, **507**, 128 (1933); **508**, 215 (1934); Fernholz and Chakravorty, *Ber.*, **67**, 2021 (1934).

¹⁴⁰ Guitoras, *Z. physiol. Chem.*, **214**, 89 (1933).

is one of the few compounds that can be degraded to the hormone progesterone.

Sitosterol. Anderson and co-workers¹⁴⁹ were the first to show that sitosterol is definitely a mixture, containing, according to their work, α -, β -, and γ -sitosterol.* The last of these is the least soluble, and the first, the most soluble. The comparison which Bengtsson¹⁵⁰ later made of β -sitosterol and its derivatives with the various derivatives of stigmasterol indicates that β -sitosterol is identical with 22-dihydrostigmasterol.¹⁵¹ Apparently γ -sitosterol differs from β -sitosterol merely in the stereochemical arrangement of the principal side chain.¹⁵¹ Wallis and Fernholz¹⁵¹ in 1936 were able to separate the so-called α -sitosterol into two fractions: α_1 - and α_2 -sitosterol, with the composition and physical properties shown in Table II. All the sitosterols are precipitated by digitonin.

Other Plant Sterols. Most of the other plant sterols that have been described have not been investigated enough to characterize them definitely as sterols. Cinchol,¹⁵² however, has been degraded through the stage of *epidi*hydrocinchol to the male hormone, androsterone,¹⁵³ and is, therefore, definitely a sterol. The physical properties of cinchol are very close to those usually given for crude sitosterol, and further investigation may reveal identity between one of the sitosterols and cinchol.

In the cases of α -spinasterol¹⁵⁴ and fucosterol,¹⁵⁵ the corresponding stanols and various derivatives have been prepared. Heilbron¹⁵⁵ has compared the fucostanol series with stigmastanol derivatives and has found excellent agreement in their physical properties. A comparison of the data of Larsen and Heyl¹⁵⁴ with those of Heilbron shows that α -spinastanol, also, has the same properties as stigmastanol. Should identity be established definitely, the composition of α -spinastanol

¹⁴⁹ Anderson and Shriner, *J. Am. Chem. Soc.*, **48**, 2976 (1926); Anderson, Shriner, and Burr, *ibid.*, **48**, 2987 (1926). Cf. Ruzicka and Eichenberger, *Helv. Chim. Acta*, **18**, 430 (1935).

* α -Sitosterol, $C_{28}H_{50}O$, m. p. 143–144°. $[\alpha]_D - 38.7$ ($CHCl_3$), has been isolated by Simpson and Williams, *J. Chem. Soc.*, 733 (1937) from sarsaparilla root.

¹⁵⁰ Bengtsson, *Z. physiol. Chem.*, **237**, 46 (1935).

¹⁵¹ Wallis and Fernholz, *J. Am. Chem. Soc.*, **58**, 2446 (1936).

¹⁵² Hesse, *Ann.*, **228**, 294 (1885); Liebermann, *Ber.*, **17**, 871 (1884); **18**, 1805 (1885); Windaus and Deppe, *Ber.*, **66**, 1689 (1933); Dirscherl, *Z. physiol. Chem.*, **235**, 1 (1935).

¹⁵³ Dirscherl, *ibid.*, **237**, 52, 268 (1935).

¹⁵⁴ Hart and Heyl, *J. Biol. Chem.*, **95**, 311 (1932); Heyl and Larsen, *J. Am. Chem. Soc.*, **56**, 942 (1934); Larsen and Heyl, *ibid.*, **56**, 2663 (1934); Simpson, *J. Chem. Soc.*, 730 (1937). In addition to α -spinasterol, β - and γ -sterols have been isolated. γ -Spinasterol is obtained exclusively as a glycoside. Catalytic hydrogenation converts all the spinasterols to the same spinastanol.

¹⁵⁵ Heilbron and co-workers, *J. Chem. Soc.*, 1572 (1934); 1205 (1935); 738 (1936).

must be revised upward. These two sterols are of interest since both have two nuclear double bonds, one of which is probably at C₅ : C₆ and the other not in conjugation with this double bond. The low value (−3.7) of the specific rotation of α -spinasterol has led Simpson¹⁵⁴ to suggest that this sterol may not be unsaturated at C₅ : C₆. The elucidation of the structures of these sterols may reveal new variants in the general sterol picture.

The Mycosterols

Ergosterol is the principal sterol of yeast* and fungi. In addition to ergosterol, zymosterol, C₂₇H₄₄O, α -dihydroergosterol, and the polyhydroxy sterol, cerevisterol, C₂₆H₄₄O₃, have been obtained from yeast.¹⁵⁶ Fungisterol, C₂₅H₄₄O, has been isolated from several kinds of fungi.¹⁵⁷ With the exception of ergosterol and α -dihydroergosterol, all these compounds are open to question and may not be sterols.

In general sterols are not present in bacteria, but small amounts of an unknown sterol have been demonstrated in *Azobacter chroococcum*.¹⁵⁸

Ergosterol [3(cis)-Hydroxy-24-methyl-5,7,22-cholestatriene]. Pure ergosterol was apparently first obtained by Tanret^{157,159} in 1908, although he doubtless had nearly pure preparations some time before this. The sterol attracted little attention until 1926–1927, when it was discovered that irradiation with ultra-violet light converts it into a vitamin D. Subsequent work has shown that ergosterol is the principal yeast sterol. The content in yeast varies considerably in the different species,¹⁶⁰ and is influenced greatly by the nature of the substrate on which the yeast is cultured.¹⁶¹ For years ergosterol was thought to have the formula C₂₇H₄₂O, but more recent analyses by Windaus⁹³ have definitely established the composition to be C₂₈H₄₄O. The compound is a triply unsaturated homolog of cholesterol. The structure of ergosterol (XXXIII) has been determined principally by Windaus and his school at Göttingen. Because of the importance of this sterol, the details of the investigation follow:

*A small amount of kryptosterol, m. p. 135–136°, [α]_D + 58.7 (CHCl₃), is present in yeast. This compound is of interest since it forms an insoluble digitonide but apparently is not a sterol; it appears to be isomeric with lanosterol (p. 1277) [Wieland, Pasedach, and Ballauf, *Ann.*, **529**, 68 (1937)].

¹⁵⁴ Maclean, *Biochem. J.*, **22**, 22 (1928); Callow, *ibid.*, **25**, 87 (1931); Honeywell and Bills, *J. Biol. Chem.*, **99**, 71 (1932); **103**, 515 (1933); Wieland and Kanaoka, *Ann.*, **530**, 146 (1937). For list of other yeast sterols see monograph of Lettré and Inhoffen, p. 118.

¹⁵⁷ Tanret, *Compt. rend.*, **147**, 75 (1908); *Ann. chim. phys.*, **8**, 15, 313 (1908).

¹⁵⁸ Anderson, Schoenheimer, Crowder, and Stodola, *Z. physiol. Chem.*, **237**, 40 (1935); Sifferd and Anderson, *ibid.*, **239**, 270 (1936).

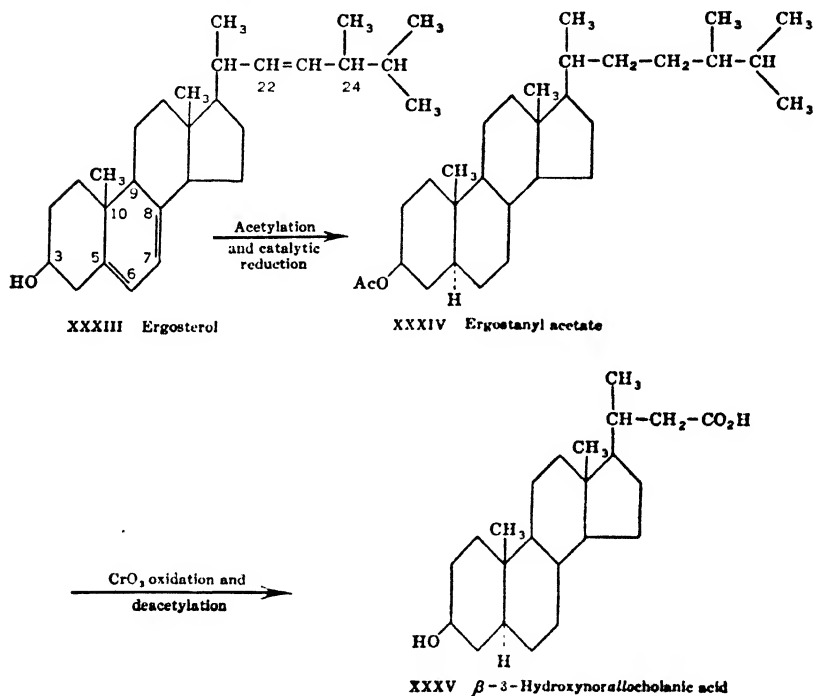
¹⁵⁹ Tanret, *Compt. rend.*, **108**, 98 (1889); *Ann. chim. phys.*, **6**, 20, 289 (1890).

¹⁶⁰ Heiduschka and Lindner, *Z. physiol. Chem.*, **181**, 16 (1929).

¹⁶¹ Bills and co-workers, *J. Biol. Chem.*, **87**, 259 (1930); **94**, 213 (1931).

1. The structure of the side chain was established by studying the action of ozone on ergosterol,¹⁶⁰ and of the products of chromic acid oxidation on a partially reduced ergosterol.⁹³ In this way a double bond at C₂₂ and a methyl group at C₂₄ were placed through the isolation of isopropylacetaldehyde and thujaketone (III), respectively.

2. For the determination of the nature of the nucleus and the position of the hydroxyl, it was necessary to have the completely saturated ergostanol. This was finally achieved by Reindel¹⁶² through catalytic



reduction of ergosterol with the Adams catalyst. From the saturated sterol the hydrocarbon ergostane was prepared. Chromic acid oxidation of ergostanyl acetate (XXXIV) gave β -3-hydroxynorallocholanic acid¹⁶³ (XXXV) of ergostane norallocholanic acid.¹⁶⁴ These two transformations determined the nature of the nucleus, placed the hydroxyl at C₃, and confirmed hypotheses based on the study of the dicarboxylic acids obtained by opening ring A.¹⁶⁵

¹⁶² Reindel and Walter, *Ann.*, **460**, 212 (1928).

¹⁶³ Fernholz and Chakravorty, *Ber.*, **67**, 2021 (1934).

¹⁶⁴ Chuang, *Ann.*, **500**, 270 (1933).

¹⁶⁵ Reindel, *Ann.*, **466**, 131 (1928).

3. The C₃ hydroxyl was shown to be part of an α,γ -system with an ethylenic bond at C₅ by a series of reactions¹⁶⁶ exactly paralleling those for cholesterol (transformation of cholesterol to cholestanetriol, etc., p. 1239).

4. The absorption spectrum of ergosterol¹⁰⁹ and the molecular refraction¹⁶⁷ indicated that a pair of double bonds was present as a conjugated system. This was supported by the fact that ergosteryl acetate forms addition products (adducts) with maleic and citraconic anhydrides.¹⁶⁸ That the adducts were formed through reaction with the conjugated system in the nucleus was established by ozone treatment⁹⁴ and reduction to dihydro compounds. From these latter, the maleic and citraconic anhydrides were removed by sublimation. The properties of the product, 22-dihydroergosteryl acetate, also indicated the presence of a conjugated system.¹⁶⁹

5. A clue to the position of the conjugated system in ergosterol was obtained by studying the action of fuming nitric acid. From this reaction mixture, toluenetetracarboxylic acid was obtained.¹⁰⁰ The reaction is explicable by assuming the conversion of a partially unsaturated to a benzenoid ring, and the wandering of a methyl group. Although the mechanism is not wholly clear, the production of this acid placed the conjugated system in ring B or C, or possibly distributed between the two, and excluded the possibility of its being contained in ring A or D.

6. Further evidence for the position of the conjugated system was obtained by studying neoergosterol. Like the other dehydrosterols, ergosterol forms a pinacol¹⁷⁰ when irradiated with visible light in the presence of a sensitizer and in the absence of oxygen. Pyrolysis of the pinacol splits off methane, and produces neoergosterol,¹⁷¹ C₂₇H₄₀O (XXXVI). The structure of neoergosterol was determined through a variety of reactions. Neoergosterol contains one reactive double bond, which is present in the side chain, since ozonization of the sterol produces methylisopropylacetaldehyde.¹⁷² This suggests that by the loss of methane the ring containing the conjugated system is converted to a benzenoid structure. Proof of this was obtained by the action of fuming nitric acid¹⁷² and by catalytic dehydrogenation¹⁷³ of

¹⁶⁶ Windaus, Inhoffen, and Reichel, *Ann.*, **510**, 248 (1934).

¹⁶⁷ Auwers and Wolter, *Nachr. Ges. Wiss. Göttingen*, 101 (1931).

¹⁶⁸ Alder, in "Handbuch der biologischen Arbeitsmethoden," Urban and Schwarzenberg, Berlin (1933), Abt. 1, Teil 2, Hälfte 2, Band 2, p. 3138.

¹⁶⁹ Windaus and Langer, *Ann.*, **508**, 105 (1933).

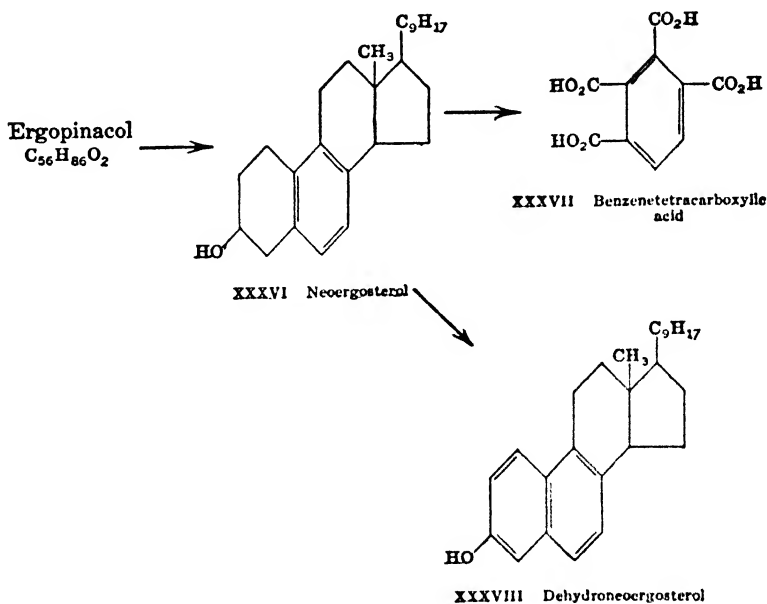
¹⁷⁰ Windaus and Borgeaud, *Ann.*, **460**, 235 (1928).

¹⁷¹ Bonstedt, *Z. physiol. Chem.*, **185**, 165 (1929).

¹⁷² Inhoffen, *Ann.*, **497**, 130 (1932).

¹⁷³ Honigmann, *Ann.*, **511**, 292 (1934).

neorgosterol. From the fuming nitric acid reaction mixture, benzenetetracarboxylic acid (XXXVII) was isolated; in neorgosterol a methyl group is therefore no longer attached to the ring, which normally is converted to toluenetetracarboxylic acid. The catalytic dehydrogenation with platinum black of neorgosterol gave a phenol, dehydroneorgosterol (XXXVIII). Zelinsky¹⁷⁴ has shown that dehydrogena-



tion of a cyclohexane ring by the action of platinum black takes place only when no quaternary carbon atom is present. Thus a methyl group is not present at C_{10} in neorgosterol. These reactions limited still further the position of the conjugated system to rings B and C.

7. The position of the conjugated system was finally placed at 5:6-7:8 by studying ergostadienetriol.¹⁷⁵ By the action of perbenzoic acid, ergosterol was converted into an oxide, which on hydrolysis gave ergostadienetriol (XXXIX), containing two secondary and one tertiary hydroxyl groups.* On reduction, the dienetriol was converted to ergostanetriol (XL), which gave the reactions of an α -glycol when tested by the lead tetra-acetate method of Criegee.¹⁷⁶ Evidently, 1,2-

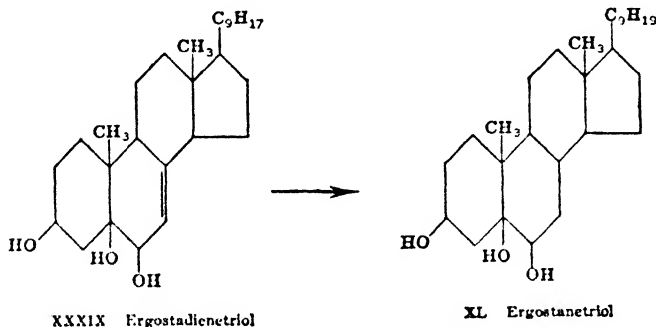
¹⁷⁴ Zelinsky, *Ber.*, **44**, 3121 (1911); **45**, 3678 (1912); **56**, 1716 (1923).

¹⁷⁵ Windaus and Lüttringhaus, *Ann.*, **481**, 119 (1930); Windaus, Inhoffen, and v. Reichel, *Ann.*, **510**, 248 (1934). Cf. Heilbron and co-workers, *J. Chem. Soc.*, 1410 (1933).

* Actually two isomers are produced as with the cholestatrienols, p. 1239.

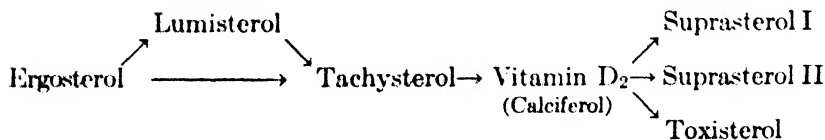
¹⁷⁶ Criegee, Kraft, and Rank, *Ann.*, **507**, 159 (1933).

addition of oxygen to the double bond at C₅ is the preliminary stage in the formation of the α -glycol. The other possible position for the conjugated system, 6:7-8:9, is definitely excluded, for only by 1,4-addition could an additional secondary and a new tertiary hydroxyl group be introduced if such a system were present, and the resulting compound would not behave like an α -glycol.



Isomerization of Ergosterol. By a variety of procedures ergosterol can be isomerized. Because of partial reduction, epimerization of the C₃-OH by treatment with sodium ethoxide cannot be employed, but can be used on certain derivatives of ergosterol. The other methods which have been used—the action of hydrogen chloride, subtraction and addition of water or hydrogen, addition and subtraction of hydrogen, and irradiation with ultra-violet light in the absence of oxygen—evidently produce a shift of the double bonds or of the hydroxyl group or both.¹⁷⁷ Alder¹⁶⁸ and Fernholz¹⁷⁸ have tabulated the several isomers, but only the changes produced by irradiation will be discussed here.

Irradiation Products of Ergosterol. The transformation that takes place when ergosterol is irradiated may be schematically represented:¹⁷⁹



The sequence of transformation may not be precisely as shown, since it

¹⁷⁷ Lettré and Inhoffen, p. 131 of monograph, give a flow sheet of these isomers.

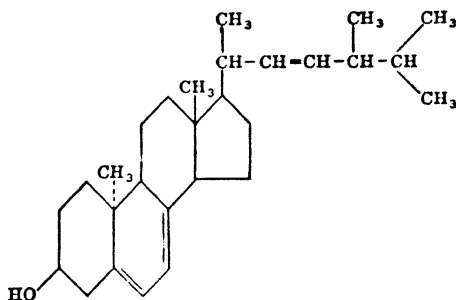
¹⁷⁸ Fernholz, *Tabulae Biologicae Periodicae*, III, 198 (1933).

¹⁷⁹ Lettré, *Ann.*, **511**, 280 (1934).

is possible that lumisterol does not represent a link in the chain of products.*

The chemical changes in this unique transformation are not entirely known, but there are enough facts to outline the nature of the reaction. All the irradiation products have the same side chain as ergosterol, for ozonization produces methylisopropylacetaldehyde in each case.¹⁰⁰ Since none of the reaction products forms an insoluble digitonide, it was at one time thought that the first change was epimerization of the C₃—OH. It is now evident that this is incorrect, and that probably the initial effect of irradiation is a spatial rearrangement of the C₁₀—CH₃. The evidence for this comes from the study of lumisterol.

Lumisterol. Of the irradiation products, only lumisterol (XLI) gives Diels' hydrocarbon when dehydrogenated with selenium,¹⁸⁰ or



XLI Lumisterol

toluenetetracarboxylic acid when oxidized with fuming nitric acid.¹⁸¹ Although lumisterol does not form a pinacol, there appears to be the same conjugated system of double bonds in ring B as in ergosterol.¹⁸² Both ergosterol¹⁸³ and lumisterol¹⁸³ are dehydrogenated by mercuric acetate to give dehydro compounds in which the new double bond is probably at C₉:C₁₁. The two compounds have the same absorption spectra, but on catalytic hydrogenation dehydrolumisterol yields perhydropyrocalciferol.¹⁸⁴ As is shown below, pyrocalciferol is a compound in which ring B has been opened and then closed by pyrolysis. Clearly lumisterol originates by a photochange in the neighborhood of

*Absorption spectrum measurements show that ultra-violet irradiation converts lumisterol more rapidly than ergosterol to tachysterol. For this reason it appears probable that lumisterol is an essential step in the transformation [Dimroth, *Ber.*, **70**, 1631 (1937)].

¹⁸⁰ Dimroth, *Ber.*, **68**, 539 (1935); Müller, *Z. physiol. Chem.*, **233**, 223 (1935).

¹⁸¹ Guiteras, Nakamiya, and Inhoffen, *Ann.*, **494**, 122 (1932).

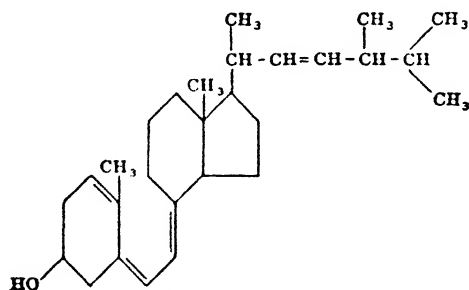
¹⁸² Heilbron, Spring, and Stewart, *J. Chem. Soc.*, 1221 (1935); Heilbron, Moffet, and Spring, *ibid.*, 411 (1937).

¹⁸³ Müller, reference 180.

¹⁸⁴ Dimroth, *Ber.*, **69**, 1123 (1936).

the linkage C_9-C_{10} , and the only change that can be reconciled with the other facts is a rearrangement involving the $C_{10}-CH_3$ group. In accord with this the *epi* compounds of lumisterol and the hydrolumisterols give insoluble digitonides.

Tachysterol. Lumisterol and tachysterol form maleic and citraconic anhydride adducts readily. Indeed, the ease of adduct formation with tachysterol is so great that its name (Gr. *tachys*, swift) is derived from this fact. Comparative hydrogenation of the citraconic anhydride adducts, or perbenzoic acid titration of the dinitrotoluyyl esters of tachysterol and dehydroergosterol, shows an equal degree of unsaturation in the two compounds.¹⁷⁹ Since dehydroergosterol contains four double bonds, tachysterol must be equally unsaturated. But tachysterol is isomeric with ergosterol, and a fourth double bond can be accommodated only by the opening of one of the rings, presumably ring B.



XLII Tachysterol
(provisional)

Structure XLII may be regarded as a tentative formulation of this "sterol."¹⁸⁵

Vitamin D. The story of vitamin D—the discovery and the chemical characterization of the antirachitic substances produced by irradiating foodstuffs or sterols with ultra-violet light—is one of the scientific classics.¹⁸⁶ In 1924 it was noted (Steenbock; Hess) that irradiation of foodstuffs with ultra-violet light produces antirachitic properties. Subsequently the fats were studied and a clue to the nature of the provitamin was obtained when it was shown (Steenbock; Hess; Rosenheim) that irradiation of the sterols resulted in very potent preparations. At first cholesterol appeared to be the precursor of the potent substance, but it was soon found (Hess; Rosenheim; Heilbron)

¹⁸⁵ Lettré and Inhoffen, p. 299 of monograph.

¹⁸⁶ Sherman and Smith, "The Vitamins," Chemical Catalog Co., New York (1931), p. 293; "Vitamins: A Survey of Present Knowledge," His Majesty's Stationery Office, London (1932), p. 73. Harris, "Vitamins in Theory and Practice," Macmillan Co., New York, 2nd Ed. (1937).

that the activity of the irradiated product depended upon the content in cholesterol of a small amount of impurity with the absorption spectrum characteristic of ergosterol. This was taken (Rosenheim and Webster; Windaus and Hess) to indicate that ergosterol was the provitamin D of cholesterol and the view seemed to be justified when finally in 1931 a very potent crystalline compound, vitamin D₂, was isolated from the irradiation products of ergosterol. For a time vitamin D₂ was thought to be identical with the natural vitamin D of fish-liver oils, but bioassays showed that their physiological properties were not the same. The discrepancy led in part to the study of the irradiation products obtained from other dehydrosterols and it is now established that the compound obtained by irradiating 7-dehydrocholesterol is identical with one of the natural vitamin D's. From the résumé above it is apparent that there are several substances with vitamin D activity which may be produced by artificial means, and from the bioassays discussed below there is evidence that fish-liver oils contain more than one natural vitamin D.

The compound now known as vitamin D₂* or calciferol was isolated almost simultaneously by Angus, Askew, Bourdillon, *et al.*,¹⁸⁷ and by Windaus and co-workers,¹⁸⁸ both groups at first obtaining a substance (calciferol, old; vitamin D₁) which was later found to be an addition product of lumisterol and vitamin D₂. By irradiating with a magnesium arc vitamin D₂, practically free of lumisterol, was obtained by Windaus, and, at the same time, the English group found that calciferol could be separated from lumisterol by crystallization of the dinitrobenzoyl esters. Vitamin D₂ has the following properties: m.p. 115–117°, $[\alpha]_D + 103$ (alc.), potency per mg. = 40,000 International antirachitic units (I.U.). Although the pure vitamin is stable for months at 37°, oil solutions in contact with air are somewhat unstable and the "half life" of an olive oil solution under these conditions is about 3 years.¹⁸⁹ Physiologically vitamin D₂ and the other compounds with vitamin D activity regulate the phosphorus-calcium metabolism, but how they function is unknown.¹⁹⁰

The other products formed by irradiation of ergosterol are not anti-rachitic, but tachysterol and toxisterol can raise a low blood-calcium

* Windaus introduced the practice of designating the different vitamins by means of a subscript.

¹⁸⁷ Askew and co-workers, *Proc. Roy. Soc. (London)*, **107B**, 76 (1930); Angus and co-workers, *ibid.*, **108B**, 340 (1931); Askew and co-workers, *ibid.*, **109B**, 488 (1932).

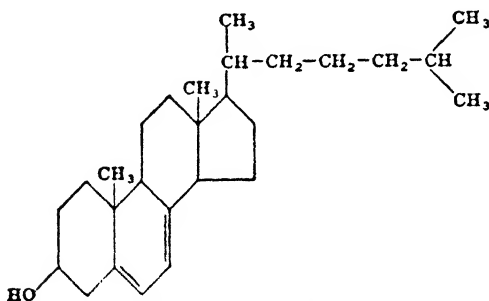
¹⁸⁸ Windaus and co-workers, *Ann.*, **489**, 252 (1931); **492**, 226 (1932). (*cf.* Lüttringhaus in "Fortschritte der physiol. Chem., 1929–1934," Verlag Chemie, Berlin (1934), p. 266.

¹⁸⁹ Bourdillon, Bruce, and Webster, *Biochem. J.*, **26**, 522 (1932).

¹⁹⁰ Bills, *Physiol. Rev.*, **15**, 1 (1935).

level to normal. The derivative dihydrotachysterol has been introduced into clinical medicine for this purpose under the designation A.T. 10 (A. T. = antitetanus).¹⁹¹

"The Multiple Nature of Vitamin D." During the period that vitamin D₂ was being studied chemically, evidence was accumulating from biological work to show that the natural vitamin D of fish oils was different from vitamin D₂, and that vitamin D activity could be conferred on cholesterol or some impurity in cholesterol.¹⁹² The differences were definitely established when Waddell¹⁹³ showed in 1934 that preparations of vitamin D₂ and of natural vitamin D, of equal potency in rats, did not have the same potency when tested on chicks, the natural being far more potent. Shortly before this it was discovered¹⁹⁴ that 22-dihydroergosterol could be converted by irradiation to a substance with antirachitic properties.* These two stimuli aroused interest again in cholesterol as a precursor of the natural vitamin D. It was soon found that cholesterol may be converted *via* 7-dehydrocholesterol¹⁹⁵ (XLIII)



XLIII 7-Dehydrocholesterol

to an antirachitic substance, vitamin D₃, with the same rat:chick assay as natural vitamin D concentrates.

The inference that vitamin D₃ is a natural vitamin D has been confirmed by the isolation of the natural vitamin itself from fish-liver oil and of the provitamin from the sterols of the skin. Prior to 1936 attempts to isolate the active constituents of fish-liver oil had led to con-

¹⁹¹ Lettré and Inhoffen, *Monograph*, p. 288.

¹⁹² Bills, *Cold Spring Harbor Symposia Quant. Biol.*, **3**, 328 (1935).

¹⁹³ Waddell, *J. Biol. Chem.*, **105**, 711 (1934).

¹⁹⁴ Windaus and Langer, *Ann.*, **508**, 105 (1933).

* It was not until the summer of 1937 that the irradiation product from 22-dihydroergosterol was obtained in pure form by Windaus and Trautmann, *Z. physiol. Chem.*, **247**, 185 (1937). Vitamin D₄, as the irradiation product is known, has the following properties: m. p. 107–108°; $[\alpha]_D^{25} + 89.3$ (alc.); potency per mg. = 20,000–30,000 I. U.; protective dose for the chick, 1γ.

¹⁹⁵ Windaus, Lettré, and Schenck, *Ann.*, **520**, 98 (1935); Windaus, Schenck, and v. Werder, *Z. physiol. Chem.*, **241**, 100 (1936).

centrates of high potency, but never to the vitamin itself. In 1936 Brockmann¹⁹⁶ isolated vitamin D₃ from concentrates of tuna-liver oil and in 1937 from halibut-liver oil.¹⁹⁷ The isolation was effected through chromatographic analysis (p. 1175) by using an aluminum hydroxide of high activity. The dyestuff Indicator Red 33 was found to be adsorbed equally with the vitamin, and thus, by adding the dye to solutions of the vitamin, the zone of adsorption could be followed very easily. A concentrated product from chromatographic analysis was converted into the 3,5-dinitrobenzoate, purified again by adsorption, and finally crystallized as the 3,5-dinitrobenzoate from acetone-methanol. In its chemical and physiological properties the natural vitamin D₃ agrees with the product obtained by irradiating 7-dehydrocholesterol. Windaus¹⁹⁸ has isolated 7-dehydrocholesterol from the sterol mixture obtained from pigskin, which was known from previous work to be relatively rich in provitamin D. Thus, from several points of attack, it is evident that 7-dehydrocholesterol is the precursor of the natural vitamin D₃, and it seems probable that the structure of the natural vitamin is analogous to that of vitamin D₂ as described below.

Certain of the rat and chick assays taken from the data of Grab¹⁹⁹ are given in Table III. Although the absolute value for these bioassays may change as a result of improved procedures, the relative values are probably correct. It is apparent from these data that vitamin D₃ from 7-dehydrocholesterol agrees most closely with the known natural vitamin D. The potency of pure vitamin D₃ is now taken to be 25,000 I.U. per mg.* The vitamin D content of fish-liver oil varies widely with species and from the ratio of rat : chick assay there is evidence that more than one natural vitamin D is present in these oils.²⁰⁰ The liver oils from the members of the percomorph family (mackerel, etc.) are far more potent than those from the halibut or cod. No good explanation has been offered for the fact that vitamin D is present in fish-liver oil and not in other liver oils, but it has been suggested that destruction of the vitamin takes place in the lungs, and, since fish lack these organs,

¹⁹⁶ Brockmann, *ibid.*, **241**, 104 (1936); Brockmann and Chen, *ibid.*, **241**, 129 (1936).

¹⁹⁷ Brockmann, *ibid.*, **245**, 96 (1937).

¹⁹⁸ Windaus and Bock, *ibid.*, **245**, 168 (1937); See also Boer, Reerink, van Wijk, and van Niekerk, *Proc. K. Akad. Wetensch. Amsterdam*, **39**, 622 (1936).

¹⁹⁹ Grab, *ibid.*, **243**, 63 (1936). For summary of methods of assay see Bomskov, "Methodik der Vitaminforschung," Thieme, Leipzig (1935), p. 171. According to Grab, the chick assays are accurate to ± 50 per cent. A more accurate bioassay is described by Massengale and Bills, *J. Nutrition*, **12**, 429 (1936).

* A very recent assay by Schenck [*Naturwissenschaften*, **25**, 159 (1937)] gives the value of 40,000 I.U. per mg. This author also lists the physical constants of vitamin D₃ as m.p. 82–84°, $[\alpha]_D + 83.3$ (acetone).

²⁰⁰ Bills, Massengale, and Imboden, *Science*, **80**, 596 (1934); Bills, *J. Am. Med. Assoc.*, **108**, 13 (1937); Bills, Massengale, Imboden, and Hall, *J. Nutrition*, **13**, 435 (1937).

TABLE III

RAT : CHICK RATIO OF VARIOUS FORMS OF VITAMIN D

Vitamin	Curative Dose, Gamma per Diem		Ratio, Rat Dose : Chick Dose
	Rat	Chick	
D ₂	0.025	0.8	1 : 32
	0.03	12.8	1 : 400
D ₃ , crystalline.....	0.06	0.2	1 : 3
concentrate.....	0.06	0.8	1 : 13
crude.....	0.15	0.3	1 : 2
Natural D, crystalline.....	0.06	0.8	1 : 12
crude concentrate.....	5.0	100	1 : 20
Irradiated 7-dehydrositosterol.....	2.0	80	1 : 40
Irradiated 22-dihydroergosterol.....	0.5	4-16	1 : 8-32

the vitamin accumulates.²⁰¹ The presence of antirachitic substances in fish is also puzzling; if the vitamin comes from the diet, then the structure of the antirachitic substances in fish oils would be related to the plant sterols rather than to the zoosterols. Actually the antirachitic substances from the plant sterols have very low potencies, for the Göttingen group has found that irradiation of 7-dehydrositosterol gives a product of low antirachitic potency²⁰² and that the product from 7-dehydrostigmasterol is inactive.²⁰³ There are probably compounds with antirachitic properties other than those described above, all of which result from the ultra-violet irradiation of dehydrosterols. Some of the possibilities that must be considered are the antirachitic substances that have been produced from cholesterol by the action of fuller's earth,²⁰⁴ butyl nitrite,¹⁹² etc. These substances may fit into or modify the general structural pattern that is described below.

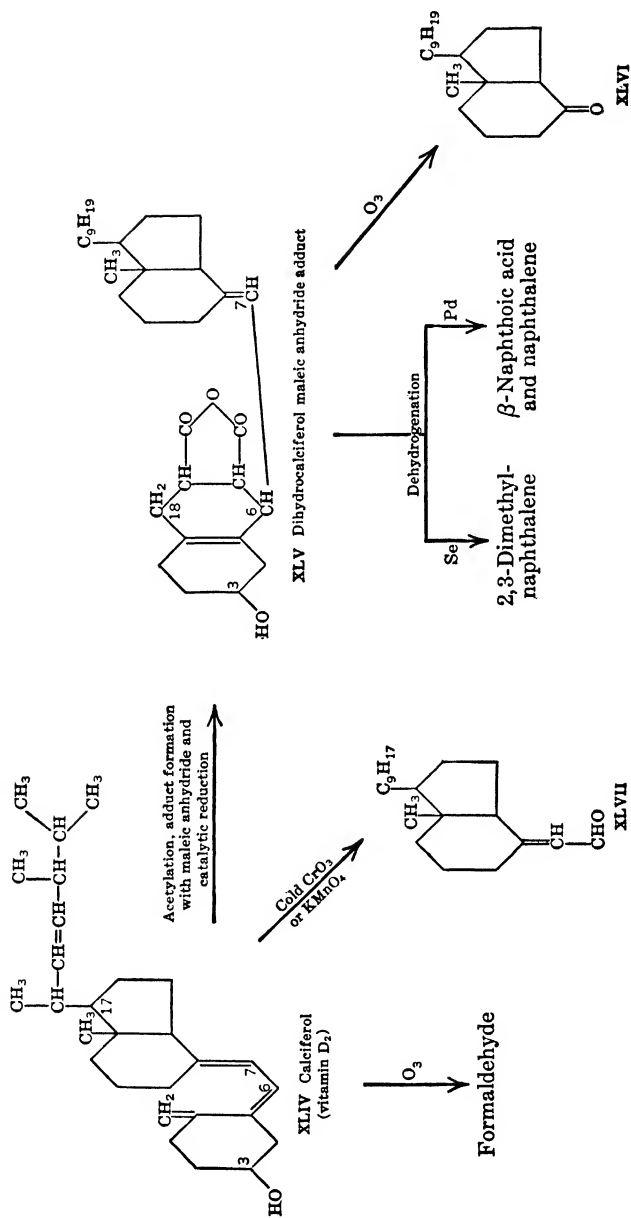
Vitamin D₂. The structure of vitamin D₂ (XLIV) has been determined by study of the products of oxidative degradation of the vitamin or of the dihydrocalciferol maleic anhydride adduct. Previous to this

²⁰¹ Coppens and Metz, *Biochem. Z.*, **266**, 169 (1933).

²⁰² Wunderlich, *Z. physiol. Chem.*, **241**, 116 (1936). See, also, Bills, *J. Am. Med. Assoc.*, **108**, 13 (1937).

²⁰³ Linsert, *ibid.*, **241**, 125 (1936).

²⁰⁴ Yoder, *J. Biol. Chem.*, **116**, 71 (1936); Eck and Thomas, *ibid.*, **119**, 621, 631 (1937).



work it was known from microcatalytic hydrogenation²⁰⁵ that vitamin D₂ contains four double bonds of which three can be detected by titration with perbenzoic acid,²⁰⁶ and that reduction with sodium and ethyl alcohol gives a dihydrovitamin that perbenzoic acid titration shows to have three double bonds.²⁰⁶ With maleic anhydride calciferyl acetate forms two isomeric α - and β -adducts which are easily reduced to dihydro compounds (XLV) in which the side chain is saturated. These vitamin and dihydrovitamin adducts are much more stable than those from ergosterol and tachysterol, and may be distilled without decomposition. Ozone, acting on the dihydro adducts, degrades them to a ketone²⁰⁷ (XLVI), C₁₉H₃₄O. The structure of this ketone is arrived at indirectly. Selenium dehydrogenation of the dihydro adduct forms 2,3-dimethylnaphthalene, and palladium dehydrogenation yields β -naphthoic acid and naphthalene. These dehydrogenation products must originate from ring A and the ring system produced in adduct formation. The production of 2,3-dimethylnaphthalene and β -naphthoic acid is particularly significant, since their formation shows the course of the adduct formation to be by addition at C₆ and C₁₈.²⁰⁷ Since the selenium dehydrogenation to 2,3-dimethylnaphthalene results in the reduction of carboxyl groups to methyl and this kind of dehydrogenation was unique at the time, the result has been checked by the study of a number of model dehydrogenations.²⁰⁸

Further evidence for structure XLIV has been obtained by direct oxidation of vitamin D₂ with cold chromic acid or permanganate to an oily aldehyde²⁰⁹ (XLVII), C₂₁H₃₄O. The semicarbazone of this aldehyde absorbs in the ultra-violet with a maximum at 275 m μ , as do other semicarbazones of α,β -unsaturated aldehydes. Under other conditions permanganate oxidation yields the Δ^{22} -unsaturated ketone corresponding to XLVI. Ozone oxidation of vitamin D₂ gives as high as 30 per cent of the theoretical formaldehyde formed by the rupture of the methylene linkage at C₁₀ : C₁₈. This last bit of evidence is not free from objection, however, since similar treatment of ergosterol gives small amounts of formaldehyde.²¹⁰

Presumably the irradiation products from the other 7-dehydrosterols are structurally related to vitamin D₂. Evidently the factors that affect antirachitic activity are the presence or absence of a double bond at C₂₂ and the nature of the group at C₂₄. Whether changes at these positions

²⁰⁵ Kuhn and Möller, *Angew. Chem.*, **47**, 145 (1934).

²⁰⁶ Windaus, Linsert, Lüttringhaus, and Weidlich, *Ann.*, **492**, 226 (1932).

²⁰⁷ Windaus and Thiele, *Ann.*, **521**, 160 (1935).

²⁰⁸ Thiele and Trautmann, *Ber.*, **68**, 2245 (1935).

²⁰⁹ Heilbron, Jones, Samant, and Spring, *J. Chem. Soc.*, 905 (1935).

²¹⁰ Windaus and Grundmann, *Ann.*, **524**, 295 (1936).

alter the rate of absorption from the intestine or determine the activity of the compounds *in vivo* is unknown.

Transformation Products of Vitamin D₂. When vitamin D₂ is heated for four hours at 180°, its potency is completely destroyed and two pyro isomers, isopyrovitamin D₂¹⁸⁸ and pyrocalciferol,¹⁸⁷ are produced. The nature of this isomerism has been examined by Windaus.²¹¹ Both these compounds are triply unsaturated and give Diels' hydrocarbon when dehydrogenated with selenium, or toluenetetracarboxylic acid when oxidized with nitric acid.¹⁸⁰ Isopyrovitamin D₂ forms an insoluble digitonide, but pyrocalciferol does not. If isopyrovitamin D₂ is dehydrogenated by treatment with mercuric acetate or perbenzoic acid, dehydroergosterol is obtained. Pyrocalciferol, on the other hand, is dehydrogenated by mercuric acetate to dehydrolumisterol. Since dehydrogenation with mercuric acetate destroys the asymmetry of C₉ through the introduction of a double bond at C₉ : C₁₁, it follows that ergosterol and isopyrocalciferol are stereochemically similar about C₁₀, but dissimilar about C₉. Lumisterol and pyrocalciferol are likewise similar at C₁₀ and dissimilar at C₉.^{*} As the previous discussion has shown, lumisterol differs from ergosterol in the steric position of the C₁₀—CH₃ group, and it is improbable that epimerization of the C₃—OH group has occurred in any of these compounds. Thus the interrelationship about the centers of asymmetry, C₉ and C₁₀, may be represented as shown below:

	C ₉	C ₁₀		C ₁₀	
Ergosterol.....	+	+	}	Dehydroergosterol.....	+
Isopyrovitamin D ₂	-	+			
Lumisterol.....	+(?)	-	}	Dehydrolumisterol.....	-
Pyrocalciferol.....	-(?)	-			

Irradiation of vitamin D₂ destroys its potency and converts it into a mixture of suprasterol I and II, and a poorly defined substance, toxisterol.²¹² Suprasterol I and II differ in that the former contains three, and the latter four, double bonds.¹⁸⁰

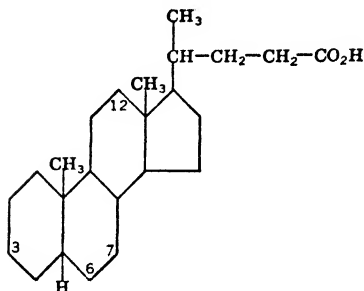
²¹¹ Windaus and Dimroth, *Ber.*, **70**, 376 (1937).

^{*} Inversion about C₉ in these pyro isomers does not interfere with the ability to undergo a photo change when irradiated with ultra-violet light. The products, photoisopyrovitamin D₂ and photopyrocalciferol, are not antirachitic, however, and the photo change may be reversed merely by heating in the absence of air. The photo products do not absorb in the ultra-violet, and Dimroth, *Ber.*, **70**, 1631 (1937), has suggested that in the photo change the conjugated system is destroyed by a shift of the 5 : 6 double bond to the 4 : 5 position.

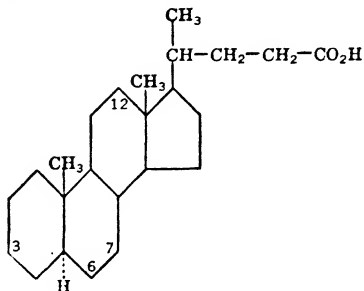
²¹² Laquer and Linsert, *Klin. Wochenschr.*, **19**, 753 (1933).

THE BILE ACIDS²¹³

Occurrence. From the bile of various species, a number of hydroxy acids have been isolated. These acids are derivatives of cholan (I) acid, and are excreted, paired with glycine or taurine, as alkali salts. In Herbivora the glycocholates, and in Carnivora the taurocholates, predominate, but the nature of the conjugation depends more on species



I Cholan acid



II Allocholan acid

than on diet.²¹⁴ The derived bile acids obtained by degrading sterols, etc., are derivatives of either cholan acid, or the stereoisomer, allocholan acid (II). The properties and sources of the important natural and derived bile acids are listed in Table IV; the data are taken largely from the extensive tabulation of Dane.²¹⁵ As the table shows, there is variation of bile acid with species, but, because of lack of suitable biles, only cholic, desoxycholic, and α -hyodesoxycholic acids are readily available.

Formation. The bile acids are the products of the synthetic activity of the liver. Apparently they are formed in this organ from amino acids and not from fats, carbohydrates, or by the degradation of sterols, although tryptophan is the only amino acid which has been shown to influence favorably the formation of the bile acids.²¹⁶ It is possible that one portion of the molecule may originate from amino acids and another from some other source. Relatively large quantities of these acids are synthesized in the liver; a dog of average size, for example, produces

²¹³ Books: Fieser; Lettré and Inhoffen; Shimizu, "Über die Chemie und Physiologie der Gallensäuren," Muramoto, Okayama (1935). Reviews: Dane, in "Fortschritte der physiol. Chem., 1929-1934," Verlag Chemie, Berlin, (1934), p. 46; Wieland, *Ber.*, **67**, 27 (1934); Sobotka, *Chem. Rev.*, **15**, 312 (1934).

²¹⁴ Cf. Shimizu, reference 213 pp. 47 *et seq.*

²¹⁵ Dane, *Tabulae Biologicae Periodicae*, **III**, 58 (1933).

²¹⁶ Whipple and co-workers, *J. Biol. Chem.*, **80**, 658, 671, 685 (1928); **89**, 689, 705 (1930); Thannhauser and co-workers, *Arch. expul. Path. Pharmacol.*, **130**, 292, 308 (1928); Jenke, *ibid.*, **163**, 175 (1932); Schindel, *ibid.*, **166**, 36 (1932); Schoenheimer, Rittenberg, Berg, and Rousselot, *J. Biol. Chem.*, **115**, 635 (1936).

about 1.5 g. per day. Conjugation with taurine or glycine appears to be a detoxication process, since the free bile acids are quite toxic.²¹⁷ One of the properties of the bile acids that appears to be harmful is the ability to hemolyze red blood corpuscles in low concentration.

Isolation. In the bile there probably are some unpaired bile salts, but the paired compounds predominate. The conjugated acids may be isolated in an impure state by salting out, or dried bile may be recrystallized from absolute alcohol. The products obtained in this way are usually mixtures from which the conjugated bile salts cannot be resolved readily; saponification of such a mixture with alcoholic alkali splits the peptide linkages to give the free acids. From these hydrolytic products the individual bile acids can be isolated in crystalline form in combination with a molecule of solvent, which is lost on drying *in vacuo*. The pure acids are powders or microcrystalline.

Generally there is one bile acid predominantly present in a given bile, and this is the one isolated by the above procedure. To obtain the other bile acids the Wieland school has developed the technique of extracting an ethereal solution of the residues with a series of buffer solutions. By employing a number of solutions of varying pH a fairly clean separation can be made, but, for the most part, the acids that are present in small amounts have not been isolated or studied. The bile acids of ox bile, however, have been studied more thoroughly than those of any other bile.

Apparently all the natural bile acids are hydroxylated at C₃, and in general they are spatially alike about this center of asymmetry. From hog bile, however, α - and β -hyodesoxycholic (3,6-dihydroxycholanolic) acids have been isolated, and these two acids appear to be isomeric about C₃.^{217a} It is possible that there may be a further example of this epimerism in the α - and β -lagodesoxycholic acids (p. 1312), but the large difference in the specific rotations of the two acids makes this seem improbable. Similarly, the monohydroxy acids, lithocholic* and isolithocholic acids, may also be epimeric at C₃, but here the values for the specific rotation indicate that they are position rather than space isomers (*cf.* structure and optical rotation, p. 1261).

Nomenclature. The names that are commonly used for the bile acids frequently contain a prefix that shows the source of the acid. The application of this terminology is apparent from Table IV. Systemati-

²¹⁷ Horrall, *Physiol. Rev.*, **11**, 122 (1931); Strain and Marsh, *Am. J. Physiol.*, **115**, 82 (1936).

^{217a} Kimura, *Z. physiol. Chem.*, **248**, 280 (1937).

* This important natural acid is difficult to obtain. It is present in cattle gallstones [Fischer, *Z. physiol. Chem.*, **73**, 204 (1911)], and in the gallstones of the hog [Schoenheimer and Johnston, *J. Biol. Chem.*, **120**, 499 (1937)], but the content of lithocholic acid in cattle gallstones is low, and hog gallstones are small and very rare.

TABLE IV
 THE NATURAL AND DERIVED BILE ACIDS *

Bile Acid	Position of Attachment of Hydroxyl Groups †	M. P., ° C.	[α] _D (Alcohol)	Bile Source ‡ or Derivation
<i>Cholanic Acids, C₂₄H₄₀O₂</i>				
Allocholanic.....	170	+22.2 (CHCl ₃)	α -Hyodesoxycholic
Bufocholanic.....	236	-20.3	Bufoodesoxycholic
Cholanic.....	164	+21.6	Cholic, desoxycholic, etc.
<i>Monohydroxy Acids, C₂₄H₄₀O₃</i>				
3-Hydroxyallocholanic....	3(<i>trans</i>)	208-210	Hyodesoxycholic
β -3-Hydroxyallocholanic..	3(<i>cis</i>)	218	Hyodesoxycholic, dihydrocholesterol
6-Hydroxyallocholanic....	6	228	Hyodesoxycholic
Lithocholic.....	3(<i>trans</i>)	185-186	+32.7	Man, ox, cholic
β -3-Hydroxycholanic.....	3(<i>cis</i>)	176-177	+25.3	Lithocholic, coprosterol
7-Hydroxycholanic.....	7	96-102	7,12-Dihydroxycholanic
12-Hydroxycholanic.....	12	90-95	7,12-Dihydroxycholanic
Isolithocholic.....	?	185	+94.3	Goose (T), hen (T)
<i>Dihydroxy Acids, C₂₄H₄₀O₄</i>				
Bufoodesoxycholic.....	3, ?	197	+37	Toad
Chenodesoxycholic.....	3(<i>trans</i>), 7(<i>trans</i>)	140	+11.1	Goose (T), hen, man, ox, cholic
(anthropo-, gallo-)	7(<i>trans</i>)			
Desoxycholic.....	3(<i>trans</i>), 12	176.5	+55	Man (G and T), ox (G), sheep, dog, goat (G and T), rabbit (G), deer, cholic
7,12-Dihydroxycholanic..	7(<i>trans</i>), 12	226-227	Cholic
β -7,12-Dihydroxycholanic	7(<i>cis</i>), 12	208	Cholic
α -Hyodesoxycholic.....	3(<i>trans</i>), 6	196	+ 8.4	Pig (G), hippopotamus
β -Hyodesoxycholic.....	3(<i>cis</i>), 6	189-190	+ 5.1	Pig
Allohyodesoxycholic.....	3, 6	274	α -Hyodesoxycholic
α -Lagodesoxycholic.....	3(<i>trans</i>), 12	156-157	+80.4	Rabbit (G)
β -Lagodesoxycholic.....	?, ?	213	+37.4	Rabbit (G)
Ursodesoxycholic.....	3(<i>trans</i>), 7(<i>cis</i>)	203	+57.07	Polar bear (T)
<i>Trihydroxy Acids, C₂₄H₄₀O₅</i>				
Cholic (cholalic).....	3(<i>trans</i>), 7(<i>trans</i>), 12	196-198	+37	Man, ox, sheep, dog (T), goat, snake (T), fish (T) (Apparently present in most species.)
Nutriacholic.....	?, ?, ?	198	Otter (G)
β -Phocaecholic.....	3, 7, 23	222-232	+25.3	Walrus (T), seal (T)
<i>Tetrahydroxy Acids, C₂₄H₄₀O₆</i>				
3,7,8,12-Tetrahydroxycholanic.....	3, 7, 8, 12	223-225	Rabbit (?)

* Data from Shimizu²¹⁸ and Dane.²¹⁵† The C₂-OH and C₇-OH groups are referred to C₁₀-CH₃. The allocholanic acids are shown by the prefix "allo"; with the exception of the "bufo" acids, all the other acids are derivatives of cholanic acid.

‡ The letters in parentheses indicate the type of conjugation (G = glycine, T = taurine) in the different species.

cally the acids are named as derivatives of cholanic acid or its stereoisomer, *allocholanic acid*, but no system has been developed for the degradation products formed by oxidation. These compounds must be referred to by the colorful names which were given them when their structures were but partially known. Certain general rules are followed, however. The tricarboxylic or ketotricarboxylic acids formed by opening ring A at C₃—C₄ are known as bilianic acids; the several bilianic acids are differentiated by means of a prefix describing the parent compound—thus lithocholic acid gives lithobilianic acid (p. 1245). Opening of ring A at C₂—C₃ leads to the formation of isobilianic acids. Thilobilianic acid is the name applied to the tricarboxylic acids obtained by oxidative cleavage of ring B. The etiobilianic acids are formed by opening ring D after the C₁₇ side chain has been completely removed (*cf.* p. 1243). The names of several other types are given in the preceding and subsequent sections.

The Nuclear Hydroxyl Groups. The bile acids may be hydroxylated in the nucleus at positions C₃, C₇, and C₁₂, or in the side chain at C₂₃. In the natural bile acids the C₃—OH is *trans* to the C₁₀—CH₃; therefore these acids are related to *epicoprosterol*. The spatial configuration of the C₃—OH has been established by the oxidative degradation of *epicoprosterol* to lithocholic acid;²¹⁸ by the production of lithocholic acid from β -3-hydroxy-5-cholenic acid,²¹⁹ a product of the oxidative degradation of dibromcholesterol; by the conversion of lithocholic acid to a number of degradation products of *epicoprosterol*;²²⁰ and by the behavior of lithocholic esters and their epimers toward digitonin.²²¹ In the last instance it was found that lithocholic esters do not give insoluble precipitates with digitonin, but that their epimers, formed by catalytic hydrogenation of 3-ketocholanic acid in acid media, or by the rearrangement of lithocholic acid with sodium ethoxide, do give insoluble digitonides. Since many of the bile acids have been converted to lithocholic acid, it follows that the C₃—OH is *trans* to the C₁₀—CH₃ in them, also. The liver appears to have the ability of forming the compounds with the C₃—OH *trans* to the C₁₀—CH₃; in contrast, the kidney or other tissues produce a *cis* configuration, since 3,7,12-triketocholanic acid,* when injected subcutaneously in toads, is eliminated

²¹⁸ Ruzicka and Goldberg, *Helv. Chim. Acta*, **18**, 668 (1935).

²¹⁹ Schoenheimer and Berliner, *J. Biol. Chem.*, **115**, 19 (1936).

²²⁰ Reindel and Niederländer, *Ann.*, **522**, 218 (1936).

²²¹ Reindel and Niederländer, *Ber.*, **68**, 1243 (1935).

* The Japanese group at Okayama view the conversion of this ketonic acid to epimeric hydroxy acids as an indication that the bile acids originate from the sterols. Although this can be questioned, the conversion does suggest that in the biological synthesis of the bile acids there may be an intermediary ketone stage. The isolation of 3-hydroxy-6-

to a small extent in the urine as β -3-hydroxy-7,12-diketocholelanic acid.²²²

From the work of Lettré (p. 1261), the steric position of the C₇—OH in cholic and chenodesoxycholic acids is known to be *trans* to the C₁₀—CH₃ group. Ursodesoxycholic acid, the characteristic bile acid of the polar bear, is stereoisomeric at the C₇—OH with chenodesoxycholic acid; the C₇—OH in ursodesoxycholic acid is therefore *cis* to the C₁₀—CH₃.²²³ The spatial configuration of the hydroxyl at C₆ in hyodesoxycholic acid has not been investigated. The C₁₂—OH, present in several of the bile acids, is probably in a *trans* position with respect to the adjoining C₁₃—CH₃, since catalytic hydrogenation in glacial acetic acid of dehydrocholic (3,7,12-triketocholelanic) acid or of dehydrodesoxycholic acid returns the original bile acids²²⁴ (v. Auwers-Skita's rule, p. 1257).

There are marked differences in the reactivity of the several hydroxyl groups. Based on the ease of esterification and dehydrogenation, the reactivity of the hydroxyl hydrogen is in the order C₇ > C₃ > C₁₂.²²⁵ An hydroxyl group at C₆ is more reactive than one at C₃,²²⁶ but for lack of suitable compounds this hydroxyl cannot be compared with other positions. Deacetylation of the acetyl compounds proceeds more readily at C₃ than at C₇.²²⁷

Similar differences in reactivity are present in the dehydro (keto) bile acids. Catalytic hydrogenation of the carbonyl groups proceeds most easily at C₃, and least at C₁₂, with the C₆ and C₇ carbonyl groups intermediate in reactivity.^{225, 228} With Clemmensen reduction only the C₃ carbonyl can be reduced.^{225, 228} Thus the order of reactivity in hydrogenations becomes C₃ > C₇ > C₁₂.

The characteristic bitter taste of bile is due to the bile acids. This taste is a function of the degree of hydroxylation, for the mono- and dihydroxy acids are tasteless. Conjugation with glycine or taurine enhances and modifies the taste considerably, producing in many instances an initial sweet taste which is rapidly replaced by bitter.

When the bile acids are heated *in vacuo*, dehydration occurs. This

ketoallocholelanic acid from hog bile (p. 1311) and of 3-hydroxy-7-ketocholelanic acid, m. p. 201–202°, $[\alpha]_D -27.3$, from guinea pig bile by Iwai, *Z. physiol. Chem.*, **248**, 65 (1937), may be regarded as supporting this hypothesis.

²²² Yamasaki and Kyogoku, *Z. physiol. Chem.*, **235**, 43 (1935); Kyogoku, *ibid.* **246**, 99 (1937).

²²³ Iwasaki, *ibid.*, **244**, 181 (1936).

²²⁴ Borsche and Feske, *ibid.*, **176**, 109 (1928).

²²⁵ Wieland and Dane, *ibid.*, **210**, 268 (1932).

²²⁶ Wieland, Dane, and Martius, *ibid.*, **215**, 15 (1933).

²²⁷ Wieland and Kapitel, *ibid.*, **213**, 269 (1932).

²²⁸ Kawai, *ibid.*, **214**, 71 (1933).

probably takes place in part through the stage of lactones, since the acetyl derivatives are easily converted to unsaturated compounds. Because lactone formation is determined primarily by the position of an hydroxyl with respect to the carboxyl group, the opportunities for such structures are far greater in the degradation products than in the parent compounds themselves.

The Unsaturated Bile Acids. The products of complete dehydration of the bile acids are mixtures of the isomeric unsaturated acids.²²⁹ The physical properties of certain of these unsaturated acids are given in Table V. The course of the dehydration is illustrated by the products formed from lithocholic [3(*trans*)-hydroxycholan] acid.²³⁰ The mixture obtained on dehydration contains about nine parts of an α -acid and one part of a higher melting β -acid. The lower melting α -lithocholenic acid is probably unsaturated at C₂ : C₃, and the β -lithocholenic acid at C₃ : C₄. Separation of the mixture is effected by bromination and crystallization of the dibromides. In this way, three dibromides are obtained, one melting at 171°, another at 233°, and the third at 240°. The two melting at 171° and 233° give α -lithocholenic acid when debrominated with zinc; these two dibromides are probably epimers in which the bromine is *cis*, *cis* in one, and *trans*, *cis* in the other.

The removal of water from ring B takes place largely through the splitting off of an hydroxyl with an adjacent tertiary hydrogen. An example of this is the dehydration of 6-hydroxyallocholan acid to a levorotatory compound, which probably is unsaturated at C₅ : C₆. In ring C with the hydroxyl group at C₁₂, dehydration can give but one product, as C₁₃ is quaternary.

Dehydrating agents such as zinc chloride and sulfuric acid remove water from ring B of cholic (3,7,12-trihydroxycholan) acid to give apocholic acid and a small amount of an isomer, dihydroxycholenic acid.²³¹ The latter can be hydrogenated to desoxycholic acid, but apocholic acid cannot be reduced catalytically. Treatment of apocholic acid with hydrochloric acid partially rearranges it to an isodihydroxycholenic acid, which is also obtainable from cholic acid by the action of hydrogen chloride; isodihydroxycholenic acid cannot be reduced catalytically. Evidently the isomers result from a rearrangement of a double bond about C₈, or from C₈ to a neighboring carbon atom. In a general way, there appears to be an analogy between these compounds and the α -stenols (p. 1271).

²²⁹ Shimizu, Oda and Makino, *ibid.*, **213**, 136 (1932).

²³⁰ Wieland, Kraus, Keller, and Ottawa, *ibid.*, **241**, 47 (1936).

²³¹ Boedeker, *Ber.*, **53**, 1852 (1920); Boedeker and Volk, *Ber.*, **54**, 2489 (1921); Borsche and Todd, *Z. physiol. Chem.*, **197**, 173 (1931); Wieland and Dane, *ibid.*, **212**, 263, (1932); Yamasaki, *ibid.*, **220**, 42 (1933); **233**, 10 (1935).

Bromine dehydrogenates apocholic acid to a mixture of two isomeric acids, α - and β -dihydroxycholadienic acids.²³² The mechanism is addition of bromine followed by spontaneous loss of hydrogen bromide. The two acids may be produced from apocholic acid by the action of perbenzoic acid to give the α -dienic, or selenium dioxide to form the β -dienic acid.²³³ Both these dienic acids can be partially hydrogenated, the α -dienic acid yielding apocholic acid, and the β -dienic acid an isomeric β -apocholic acid which may be stereoisomeric about C₉.

Isodihydroxycholenic acid, when dehydrogenated with bromine, gives a dienic acid differing from the known isomers, and hydrogenation of the dienic acid likewise gives an unidentifiable product. This choladienic acid has a remarkable property—it is extracted from ether by 30 per cent hydrochloric acid.²³⁴

TABLE V
THE UNSATURATED BILE ACIDS

Acid	Formula	M. P., ° C.	$[\alpha]_D$ (Alcohol)
α -Lithocholenic (Δ^2 ?).....	C ₂₄ H ₃₈ O ₂	156	+16.3
β -Lithocholenic (Δ^3 ?).....	C ₂₄ H ₃₈ O ₂	160	+18.7
5-Cholenic (?).....	C ₂₄ H ₃₈ O ₂	160	-66.8
β -3-Hydroxy-5-cholenic.....	C ₂₄ H ₃₈ O ₃	241-242	
Apocholic (3,12-dihydroxy- $\Delta^{8,14}$?).....	C ₂₄ H ₃₈ O ₄	173-174	+45.5
Dihydroxycholenic (3,12-dihydroxy- $\Delta^{14,15}$?).....	C ₂₄ H ₃₈ O ₄	255-256	+57.6
Isodihydroxycholenic.....	C ₂₄ H ₃₈ O ₄	198	+5.9
α -Dihydroxycholadienic ($\Delta^{7,8-14,15}$?).....	C ₂₄ H ₃₆ O ₄	252-255	-35.5
β -Dihydroxycholadienic ($\Delta^{8,9-14,15}$?).....	C ₂₄ H ₃₆ O ₄	253-255	+71

The Color Reactions of the Bile Acids. The unsaturated acids are probably formed in the course of the color reactions that are given by the bile acids. The most important of these color reactions follow:

*The Pettenkofer Reaction.*²³⁵ A solution of a bile salt is mixed with a few drops of sugar solution, treated with concentrated sulfuric acid, and shaken at 70°. A red color develops and quickly passes through purple-red to blue-red. Many modifications have been proposed, particularly the use of furfural in place of sugar.²³⁶ The reaction is not given

²³² Wieland and Deulofeu, *ibid.*, **198**, 127 (1931).

²³³ Callow, *J. Chem. Soc.*, 462 (1936).

²³⁴ Wieland, Dietz, and Ottawa, *Z. physiol. Chem.*, **244**, 194 (1936).

²³⁵ Pettenkofer, *Ann.*, **52**, 90 (1844).

²³⁶ Mylius, *Z. physiol. Chem.*, **11**, 492 (1887); Gregory and Pascoe, *J. Biol. Chem.*, **83**, 35 (1929); Reinhold and Wilson, *ibid.*, **96**, 637 (1932).

by desoxycholic acid or by the keto acids; consequently, an hydroxyl group in ring B is apparently necessary for the color change.

*The Hammarsten Reaction.*²³⁷ When powdered cholic acid is added to 25 per cent hydrochloric acid, a violet color develops which gradually passes through green to yellow. The reaction is given only by cholic and apocholic acid or their derivatives.

The Liebermann Reaction. This reaction is carried out as with the sterols (p. 1272). The solid acids are employed, since the bile acids are insoluble in chloroform. The color produced varies with the bile acid.

Transformations of the Nucleus. The nuclear hydroxyl groups weaken the several rings at the point of attachment, and, by the proper selection of oxidizing agent, stepwise degradation may be effected. Cold chromic acid produces the least change, converting the carbinol groups to carbonyl to form the dehydro acids,²³⁸ while concentrated nitric acid and potassium permanganate open the rings. The possibilities of such transformations are illustrated in the degradation of desoxybilianic acid (p. 1245). Concentrated nitric acid acting over a period of time on this acid not only opens ring C, but also probably oxidizes the C₁₀ methyl group to carboxyl, forming chollepidanic acid²³⁹ (Gr., *lepis* = scale) (III). Less vigorous treatment with nitric acid merely opens ring C to produce choloidanic acid²⁴⁰ (IV). Pyrocholoidanic acid (V), the pyrolytic product from IV, on oxidation first with permanganate and then with nitric acid, passes through the stage of prosolanelllic acid (VI) to solanelllic acid²⁴¹ (*solus anellus* = one ring) (VII); pyrosolanelllic acid (VIII) in turn yields biloidanic acid²⁴² (IX). The last may be produced more easily by the action of mixed acids on bilianic acid²⁴³ (XI), the product of partial nitric acid oxidation of dehydrocholic acid (X). Bilianic acid when oxidized with permanganate suffers oxidation in ring B to give a triketo acid (XII) which, when treated with acid, undergoes a benzilic acid rearrangement to form cilianic acid²⁴⁴ (XIII).

Chromic acid oxidation in the cold of α -hyodesoxycholic acid gives the dehydro acid, 3,6-diketochoLANic acid, which is readily isomerized to the *allo* series by warming with strong acids or alkalis.²⁴⁵ The resulting 3,6-diketo*allo*choLANic (β -dehydrohyo-) acid serves as a source of a

²³⁷ Hammarsten, *Z. physiol. Chem.*, **61**, 495 (1909); Yamasaki, *ibid.*, **220**, 42 (1933).

²³⁸ Hammarsten, *Ber.*, **14**, 71 (1881).

²³⁹ Wieland and Kraft, *Z. physiol. Chem.*, **211**, 203 (1932).

²⁴⁰ Wieland and Kulenkampff, *ibid.*, **108**, 306 (1920).

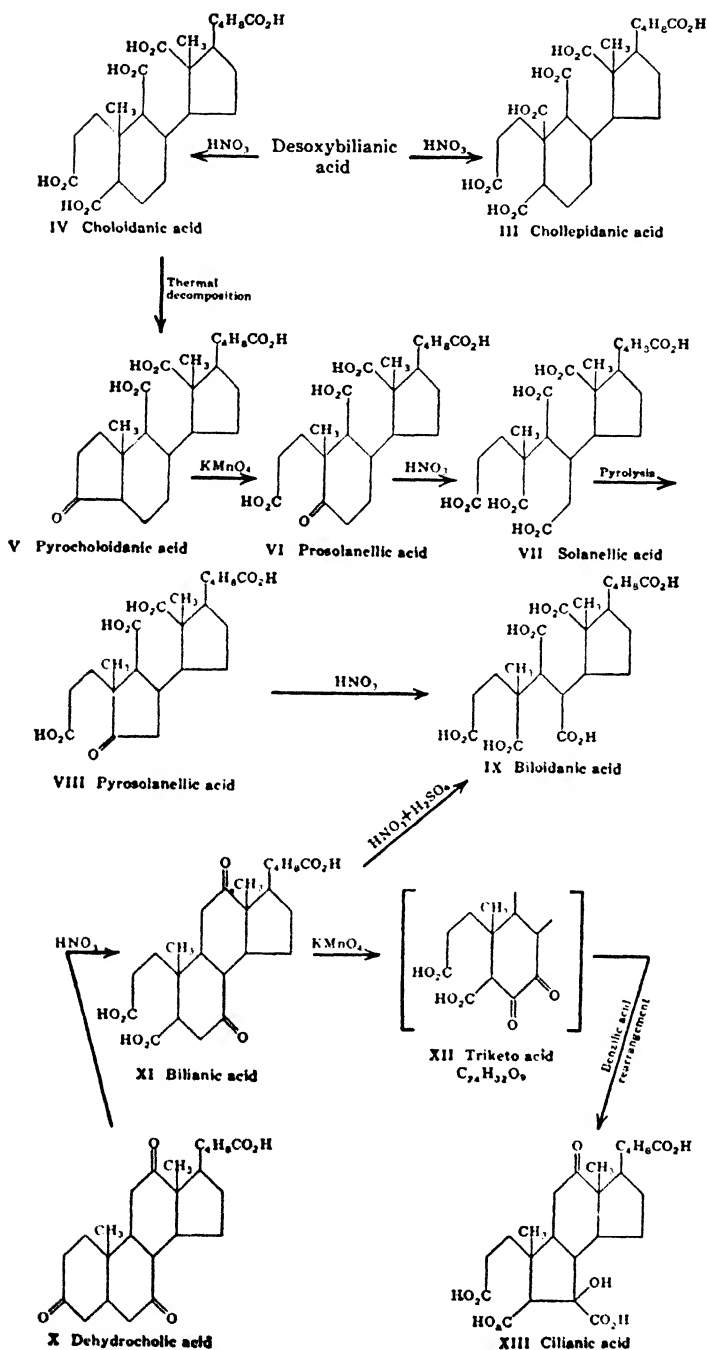
²⁴¹ Wieland and Schulenburg, *ibid.*, **114**, 167 (1921).

²⁴² Schenck, *ibid.*, **110**, 167 (1920); **112**, 38 (1921).

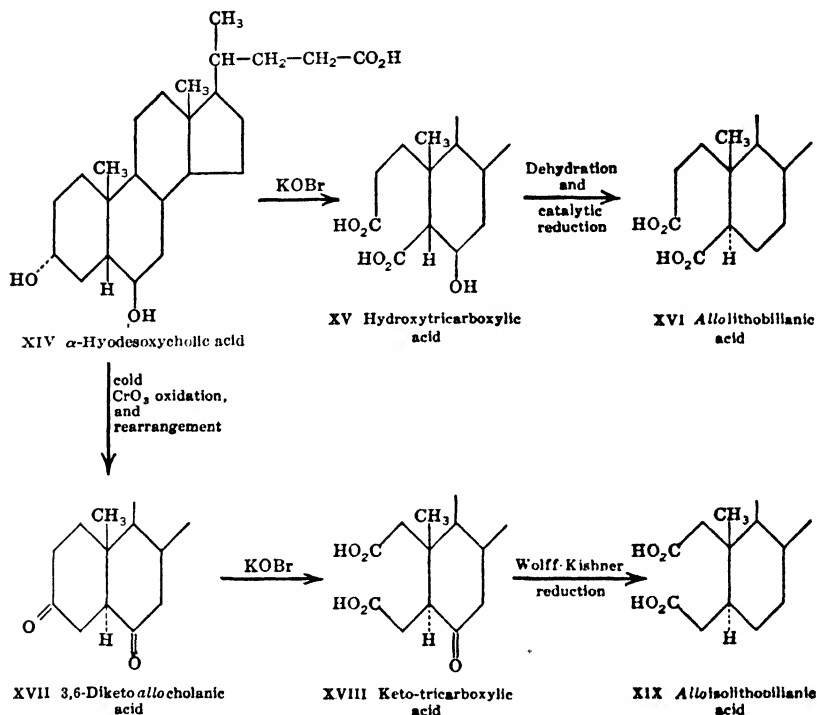
²⁴³ Wieland and Schlichting, *ibid.*, **119**, 76 (1922).

²⁴⁴ Schenck, *ibid.*, **87**, 59 (1913); **242**, 81 (1930); **244**, 245, (1930).

²⁴⁵ Windaus and Bohne, *Ann.*, **433**, 278 (1923); Windaus, *Ann.*, **447**, 233 (1926); Shibuya and Miki, *Z. physiol. Chem.*, **206**, 279 (1932).



variety of *allo* derivatives. α -Hyodesoxycholic acid (XIV) is converted by the action of hypobromite to an hydroxytricarboxylic acid (XV) which may be reduced by the action of hydrogen iodide to lithobilianic acid. Treatment of the hydroxytricarboxylic acid with sodium ethoxide dehydrates it to lithobilienic acid, and this acid is converted by catalytic hydrogenation to *allolithobilianic* acid (XVI). The isomeric *allo*-isolithobilianic acid (XIX) is obtained from 3,6-diketo*allo*cholanic acid



(XVII) by permanganate or hypobromite oxidation to a 6-ketotricarboxylic acid (XVIII), which is then reduced by the Wolff-Kishner method to the desired *allo* acid. As has been cited previously, these isomeric bilianic acids played an important part in the establishment of the spatial configuration about C_5 (p. 1253).

Bufocholanic Acid. From the bile of the toad, the specific dihydroxy bile acid, bufodesoxycholic, has been isolated;²⁴⁶ it differs stereochemically from the others. The corresponding dehydro acid can be rearranged like dehydrohyodesoxycholic acid, but the bufocholanic acid produced by Clemmensen reduction of these two dehydro acids is not iden-

²⁴⁶ Okamura, *J. Biochem. (Japan)*, **8**, 351 (1928); **10**, 5 (1928); **11**, 103 (1929).

tical with either cholanic or *allocholanic* acid. On opening ring A of these bufodehydro acids by the action of hypobromite, ketotricarboxylic acids are produced, which, on Clemmensen reduction, are converted to *allolithobilianic* acid. From this evidence, one of the hydroxyl groups is placed at C₃, but the position of the other hydroxyl is uncertain. Obviously this second hydroxyl group is adjacent to one of the centers of asymmetry.

The C₁₀—CH₃ Group. With many of the bile acids, pyrolysis in an atmosphere of carbon dioxide splits off the C₁₀—CH₃ as methane. The reaction parallels the conversion of the sterol pinacols to the norsterols. The lability of the methyl group is markedly affected by structure; for example, in apocholic acid, more than a third of the molecule suffers this type of change, but with most of the bile acids very little methane is split off.²⁴⁷

Molecular Compounds. The property of forming molecular compounds is very pronounced in the bile acids, and, as commonly obtained, they crystallize with a molecule of solvent of crystallization. One of the unique molecular compounds is the blue compound (C₂₄H₄₀O₅·I)₄·KI·H₂O, formed when an alcoholic solution of cholic acid and a solution of iodine in potassium iodide are mixed in the correct proportions.²⁴⁸

With any of a wide variety of substances, desoxycholic acid forms a series of water-soluble molecular compounds which are collectively known as the *choleic acids*. The compounds formed with the fatty acids were the first examples noted and have since received considerable study.²⁴⁹ With increasing molecular weight, the molecular ratio of bile acid : fatty acid changes from 1 : 1 with acetic acid to 8 : 1 in the case of stearic acid. The variations are shown graphically in Fig. 1 both for fatty acids and dicarboxylic acids. Since formation of choleic acids is due to coördinate valences, the compounds are conveniently described by a coördination number which expresses the number of molecules of desoxycholic acid combined with one molecule of a second substance. As the curves show, the ratio changes by steps. The significance of this is a matter of speculation, but usually there is assumed to be a packing of molecules along the lower edge of the nucleus and the side chain (*cf.* structure I) brought about by the "enhancing" effect of the hydroxyl at C₁₂. Supporting evidence for this theory is furnished by the fact that apocholic acid forms coördination compounds comparable to those

²⁴⁷ Wieland and Dane, *Z. physiol. Chem.*, **212**, 263 (1932).

²⁴⁸ Mylius, *ibid.*, **11**, 306 (1887); Küster, *Z. physik. Chem.*, **16**, 156 (1895); Barger and Field, *J. Chem. Soc.*, **101**, 1404 (1912).

²⁴⁹ Wieland and Sorge, *Z. physiol. Chem.*, **97**, 1 (1916); Rheinboldt and co-workers, *Ann.*, **451**, 256 (1927); *Z. physiol. Chem.*, **180**, 180 (1928); *Ann.*, **473**, 249 (1929); Sobotka and Goldberg, *Biochem. J.*, **26**, 555 (1932).

of desoxycholic acid, while chenodesoxycholic and cholic acids do not. The cholic acids formed from enolizable ketones exhibit the peculiarity of being combined completely in the enolized form.²⁵⁰ Since functional groups are not necessary for the formation of cholic acids, these compounds have been suggested as a means of resolving racemic mixtures of optically active but inert compounds, such as hydrocarbons.²⁵⁰

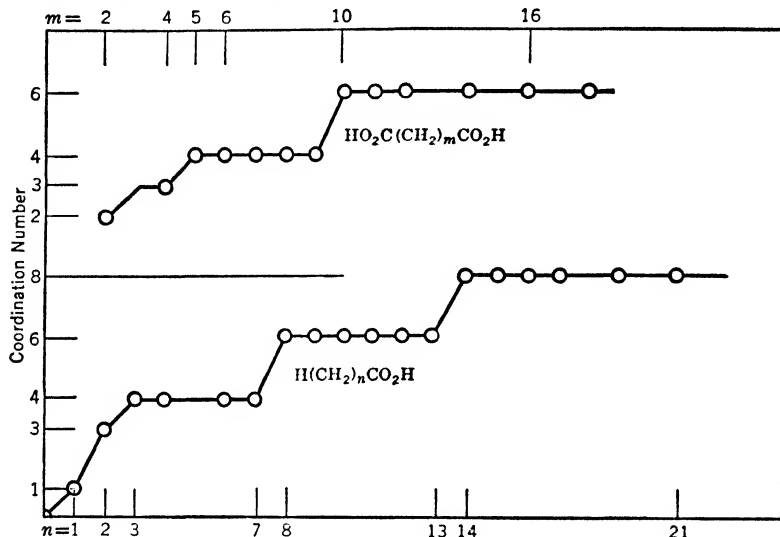


FIG. 1*

Relation between length of aliphatic chain and coordination number of the fatty acid cholic acids

The soluble salts of the bile acids lower the surface tension of water to a marked degree.²⁵¹ This property may be associated with the phenanthrene structure, for other compounds containing a phenanthrene nucleus, such as the saponins (p. 1345) and abietic acid, exhibit the same behavior.

The Natural Bile Acids. Accurate quantitative data on the distribution of the bile acids are lacking. Cholic acid, however, is the most common bile acid and is found in many species (Table IV). Beef bile has been investigated more extensively than any other. For this bile, the data of Wieland²⁵² give the following composition: Cholic acid, 5–6 per cent; desoxycholic acid, 0.6–0.8 per cent; lithocholic acid,

²⁵⁰ Sobotka and Goldberg, *ibid.*, **26**, 905 (1932); Marx and Sobotka, *J. Org. Chem.*, **1**, 275 (1936).

* From Sobotka, *Chem. Rev.*, **15**, 362 (1934). (Courtesy of the publishers).

²⁵¹ Allen, *J. Biol. Chem.*, **22**, 505 (1915).

²⁵² Wieland and Jacobi, *Z. physiol. Chem.*, **148**, 232 (1925).

0.002 per cent; and traces of chenodesoxycholic acid, sterocholic acid, (see below) and Weyland's acid (see below). The role of the various acids in metabolism is not known nor is there any explanation for the variation of acid with species. Although an important function in the absorption of fats is usually attributed to the bile acids,²⁵³ studies on bile-deprived animals show that fat absorption takes place even in the absence of bile. Dogs deprived of bile by operative procedures suffer decalcification of the bones and develop other anomalies.*

The Derived Bile Acids. Synthetic work in this group has been directed largely towards the conversion of the common bile acids to the uncommon. In addition to the preparation of the rare lithocholic²⁵⁴ and chenodesoxycholic²¹⁹ acids, a number of isomeric monohydroxy acids have been prepared. Those reported are *allo*-lithocholic²⁵⁵ [3(*trans*)-hydroxy*allo*cholanolic], 3-hydroxy*allo*cholanolic,²²⁵ 6-hydroxy*allo*cholanolic,²⁵⁵ 7-hydroxycholanolic,²²⁴ and 12-hydroxycholanolic²⁵⁶ acids. When dihydroxycholenic acid is oxidized with dilute permanganate, the tetrahydroxy acid, 3,7,8,12-tetrahydroxycholanolic acid, results.²⁵⁷ This acid may be identical with an acid isolated from rabbit bile.²⁵⁸

In the course of the oxidative degradation of the saturated sterols, a number of bile acids have become available, especially 3(*cis*)-hydroxy-5-cholenic acid, the product of oxidative degradation of dibromocholesterol. The physical characteristics and the literature of some of the other degradation products have been summarized by Reindel.²²⁰

Miscellaneous Bile Acids. In addition to the acids described above, a few others have been isolated from various sources. Wieland²⁵⁹ has described a compound from ox bile which is made up of one molecule of chenodesoxycholic acid and one molecule of 3-hydroxy-12-ketocholanolic acid. This molecular compound is known as Weyland's acid. An isomeric keto acid, 3-hydroxy-6-keto*allo*cholanolic, has been isolated from pig bile.²⁶⁰ It is uncertain whether the *allo* configuration is that of the native bile acid or whether rearrangement occurs during isolation.

²⁵³ Verzar and McDougall, "Absorption from the Intestine," Longmans, Green and Co., London (1936), pp. 158 *et seq.*

* These statements are from personal communications by W. B. Hawkins and R. G. Sinclair, both of the School of Medicine and Dentistry, University of Rochester.

²⁵⁴ Borsche and Hallwase, *Ber.*, **55**, 3318 (1922); Wieland, Dane, and Scholz, *Z. physiol. Chem.*, **211**, 206 (1932).

²⁵⁵ Wieland and Dane, *ibid.*, **212**, 41 (1932).

²⁵⁶ Wieland and Schlichting, *ibid.*, **150**, 267 (1925).

²⁵⁷ Wieland and Dane, *ibid.*, **206**, 243 (1932).

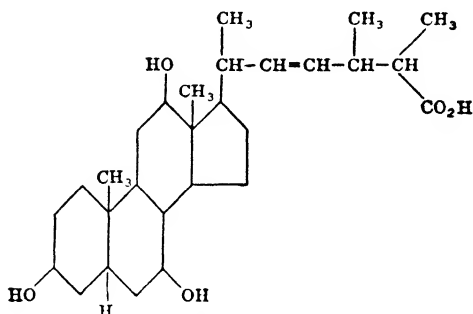
²⁵⁸ Windaus and van Schoor, *ibid.*, **173**, 312 (1928).

²⁵⁹ Wieland and Kishi, *ibid.*, **214**, 47 (1933).

²⁶⁰ Fernholz, *ibid.*, **232**, 202 (1935); Schoenheimer and Johnston, *J. Biol. Chem.*, **120**, 499 (1937).

In the bile of the rabbit, desoxycholic acid conjugated with glycine is predominately present but there are also small amounts of the isomeric acids, α - and β -lagodesoxycholic acids.²⁶¹ The α -acid gives dehydrodesoxycholic acid when it is oxidized with cold chromic acid and appears to be epimeric at C₁₂ with desoxycholic acid. It differs from the latter in its inability to form choleic acids. The β -lagodesoxycholic acid has not been converted to a known bile acid derivative as yet, and its structure is completely unsolved.

Two C₂₈ bile acids have been isolated also. The first, sterocholic acid, C₂₈H₄₆O₄, was isolated from ox bile;²⁵⁹ related compounds are apparently present in the bile of the snapping turtle.²⁶² The second



XX Trihydroxybufosterocholenic acid
(Steric position of hydroxyl groups uncertain)

C₂₈ acid, trihydroxybufosterocholenic acid (XX), C₂₈H₄₆O₅, was obtained from toad bile.²⁶³ Oxidative degradation together with Wicland degradation, has established its structure. It is interesting that the construction of the side chain of trihydroxybufosterocholenic acid should resemble so closely that of ergosterol.

Scymnol. In the bile of the shark, a sulfuric acid ester of a tetrahydroxy compound, scymnol (XXI), C₂₇H₄₈O₅, is found, rather than the usual bile acids.²⁶⁴ Oxidation of scymnol with chromic acid converts it to a triketo acid, C₂₇H₃₆O₆, demonstrating that three of the hydroxyl groups are secondary and one primary. The uncharacterized oxygen atom, both in scymnol and the triketo acid, appears to be present in an ethylene oxide ring, for the alcohol and the keto acid add hydrogen chloride, producing chlorohydrins. The chlorohydroxyketo acid formed from the triketo acid by addition of hydrogen chloride may

²⁶¹ Kishi, *Z. physiol. Chem.*, **238**, 210 (1936).

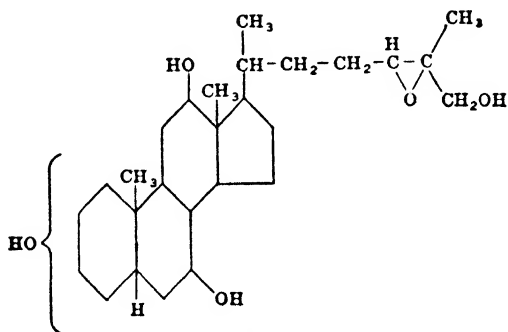
²⁶² Yamasaki and Yuuki, *ibid.*, **244**, 173 (1936).

²⁶³ Shimizu and Oda, *ibid.*, **227**, 74 (1934); Shimizu and Kazuno, *ibid.*, **239**, 67, 74 (1936); **244**, 167 (1936).

²⁶⁴ Windaus, Bergmann, and König, *ibid.*, **189**, 148 (1930).

be oxidized to a triketocholanic acid, $C_{24}H_{34}O_5$, which is not identical with 3,7,12-triketocholanic acid, but can be converted to the known 7, 12-diketocholanic acid by Clemmensen reduction.²⁶⁵ Thus three of the four hydroxyl groups are placed, and it is apparent that scymnol is one of the few natural compounds of this group in which the C_3-OH is absent.

The Conjugated Bile Acids. Some conjugated bile acids can be obtained pure by recrystallization from absolute alcohol of dried bile or of the products obtained by salting out.²⁶⁶ In general, however, this is



XXI Scymnol

not a satisfactory procedure, and the conjugated compounds are best prepared by synthetic means from the free acids. The method of Cortese and Bauman²⁶⁷ appears to be the only satisfactory one. In their procedure the hydroxyl groups are protected by treatment with formic acid and the formyl esters coupled with glycine or taurine by the usual Schotten-Baumann process. The conjugated acids are more acidic and more soluble than the free bile acids. After the discovery of the *choleic acids*, physiologists suggested the formation of such compounds as a mechanism for the absorption of fats from the intestine.²⁵³ Since neither the glyco²⁶⁷ nor the tauro²⁶⁸ compounds of desoxycholic acid form *choleic acids*, such a mechanism cannot be seriously considered.

²⁶⁵ Tschesche, *ibid.*, **203**, 263 (1931).

²⁶⁶ Hammarsten, "Abderhalden's Handbuch der biologischen Arbeitsmethoden," Urban and Schwarzenberg, Berlin (1925), 1, VI, 211.

²⁶⁷ Cortese and Bauman, *J. Am. Chem. Soc.*, **57**, 1393 (1935); *J. Biol. Chem.*, **113**, 779 (1936); Cortese and Bashour, *ibid.*, 119, 177 (1937).

²⁶⁸ Wieland, *Z. physiol. Chem.*, **106**, 181 (1919).

THE CARDIAC AGLUCONS AND THE TOAD POISONS²⁶⁹

A number of glycosides of plant origin and the nitrogenous venoms secreted by the parotid glands of toads possess valuable cardiotonic properties. The administration of these substances to individuals with damaged heart function results in a decrease in the rate and an increase in the intensity of the heart beat. Overdosage produces pernicious vomiting and stoppage of the heart in systolic standstill. The cardiac principles are used for other purposes in medicine, but the principal use is for their characteristic heart action. In addition to their therapeutic use, certain of the glycosides have been employed as arrow and ordeal poisons by savage tribes, particularly by the natives of Africa and the Malayan peninsula.²⁷⁰ Acid or enzymatic hydrolysis of the glycosides, or of the venoms, splits off the sugar residues, or the nitrogenous bases, to give the so-called genins (Fr., *génie*, spirit). In the case of the glycosides these hydrolytic products are also called aglucons. The free genins are sparingly soluble and are convulsive poisons rather than satisfactory heart stimulants. They are valueless medicinally.

The Cardiac Aglucons

The Cardiac Glycosides. The chief sources of the cardiac glycosides are the members of the plant orders *Apocynaceae* and *Scrophulariaceae*. Of the latter order certain genera of *Digitalis* (foxglove) furnish most of the drugs of therapeutic value. The principal glycosides, their sources, and their hydrolytic products are listed in Table VI. In this compilation the melting points have not been given, since these are more functions of the method of purification and of the rate of heating than of the glycosides themselves. In a few instances there is some uncertainty as to the nature of the sugar portion of the molecule.

The cardiac glycosides are obtained by extraction from the plant tissues indicated in Table VI. The isolation of the pure glycosides in quantity is difficult because of the low content in the plant tissues and because of the presence of other substances that materially modify the solubility relations. Most of the glycosides that have been studied are probably not the true plant glycosides, since there are enzymes in the plants that rapidly bring about a partial hydrolysis. To prevent enzymatic hydrolysis, fresh leaves may be macerated with ammonium

²⁶⁹ Books: Fieser; Lettré and Inhoffen; Stoll, "The Cardiac Glycosides," The Pharmaceutical Press, London (1937). Reviews: Jacobs, *Physiol. Rev.*, **13**, 222 (1933); Kon, "Ann. Repts. Chem. Soc. (London)," Vol. 31, p. 218 (1934); Elderfield, *Chem. Rev.*, **17**, 187 (1935); Tschesche, *Ergeb. Physiol.*, **38**, 31 (1936). For preparative details of the glycosides see Van Rijn, "Die Glycoside," Borntraeger, Berlin (1931).

²⁷⁰ Lewin, "Die Pfeilgifte," Barth, Leipzig (1923).

sulfate, the mixture pressed with an hydraulic press to expel water, and the glycosides extracted from the press cake with cold ethyl acetate. After evaporating this solvent in the cold, the residue is washed with ether, and the tannins and saponins removed by precipitation with neutral lead acetate. The glycosides are finally obtained by recrystallizing from a solvent like methanol, all operations being carried out at low temperatures. The digilanides A, B, and C, the purpurea glycosides, and scillaren A are representatives of the true plant glycosides.²⁷¹ The other compounds of Table VI are obtained from the dried tissues and are probably enzymatic degradation products, as has been shown to be true in a few instances. Thus, the juices of the fresh leaves have been used to convert purpurea glycoside A to digitoxin, purpurea glycoside B to gitoxin, and digilanide C to digoxin.²⁷¹ From the dried leaves the glycosides are generally extracted by means of alcohol, the tannins removed, and the products purified by precipitation and crystallization, or by partition between solvents, e.g., chloroform and water, or water-methanol. Although the pure glycosides are sparingly soluble in water, they may be extracted by this solvent in a few instances. The final purification is similar to that given above.²⁷² The preparation of the glycosides in pure form is difficult because these extraction procedures frequently give mixtures of closely allied substances. The lack of pure products, together with the difficulty of obtaining satisfactory analyses by combustion, has been a great handicap, and the empirical formulas of the glycosides have been often revised.

As many as four sugar molecules may be present in the native glycosides, and they are apparently always attached to the genin at the C₃—OH (see formula I below). The sugars are for the most part α -desoxy sugars (cymarose, digitoxose, and sarmentose), antiarose, digitalose, glucose, and rhamnose; their chemistry is discussed in Chapters 16, 17. With the exception of glucose and rhamnose, these sugars are not found elsewhere in nature. When either glucose or rhamnose is joined directly to the genin molecule, the union is much firmer than with the other sugars, and the conditions necessary for complete hydrolysis are so drastic that partial dehydration occurs with the production of anhydrogenins, e.g., hydrolysis of ouabain, uzarin, etc.

The Aglucons. With the exception of scillaridin A the aglucons may be described by the formula given in structure I. As this formulation shows, they may be regarded as derivatives of a norcholanolic acid in which the C₂₀—CH₃ has been replaced by an aldehyde group. The

²⁷¹ Stoll and Kreis, *Helv. Chim. Acta*, **16**, 1049, 1390 (1933).

²⁷² Lettré and Inhoffen, pp. 177 *et seq.* give many of the experimental details. See, also, Stoll, reference 269.

TABLE VI
THE PRINCIPAL CARDIAC GLYCOSIDES *

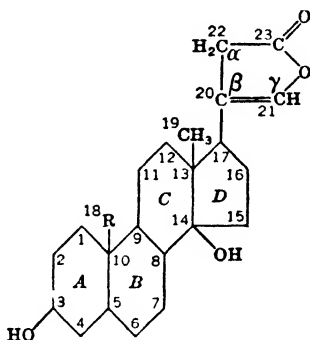
Glycoside	Formula	Plant Sources †	Hydrolytic Products	
			Genin	Sugars
<i>Apocynaceae</i>				
Ouabain	C ₂₉ H ₄₄ O ₁₂	Ouabaio tree (R) <i>Strophanthus gratus</i> (S)	Anhydroouabagenin	Rhamnose
Cymarin	C ₃₀ H ₄₄ O ₉	<i>Strophanthus kombé</i> (S)	Strophanthidin	Cymarose
Sarmentocymarín	C ₃₀ H ₄₆ O ₉	<i>Strophanthus sarmentosus</i> (S)	Sarmentogenin	Sarmentose
Oleandrin	C ₃₂ H ₄₈ O ₉	<i>Nerium oleander</i> (L)	Gitoxigenin	Oleandrose (and acetic acid)
k-Strophanthin-β	C ₃₄ H ₅₄ O ₁₄	<i>Strophanthus kombé</i> (S)	Strophanthidin	Strophanthobiose (cymarose and glucose)
Thevetin	C ₄₂ H ₆₆ O ₁₃	<i>Thevetia nerifolia</i> (S)	Anhydrothevetigenin	2 Glucose and digitalose (?)
<i>Scrophulariaceae</i>				
Digitoxin	C ₄₁ H ₆₄ O ₁₃	<i>Digitalis purpurea</i> (L)	Digitoxigenin	3 Digitoxose
Gitoxin	C ₄₁ H ₆₄ O ₁₄	<i>Digitalis purpurea</i> (L)	Gitoxigenin	3 Digitoxose
Digoxin	C ₄₁ H ₆₄ O ₁₄	<i>Digitalis lanata</i> (L)	Digoxigenin	3 Digitoxose
Purpurea glycoside A	C ₄₇ H ₇₀ O ₁₈	<i>Digitalis purpurea</i> (L)	Digitoxigenin	3 Digitoxose and 1 glucose
Purpurea glycoside B	C ₄₇ H ₇₀ O ₁₉	<i>Digitalis purpurea</i> (L)	Gitoxigenin	3 Digitoxose and 1 glucose
Diglanide A	C ₄₈ H ₇₄ O ₁₉	<i>Digitalis lanata</i> (L)	Digitoxigenin	2 Digitoxose, acetyl-digitoxose, and glucose
Diglanide B	C ₄₈ H ₇₄ O ₂₀	<i>Digitalis lanata</i> (L)	Gitoxigenin	2 Digitoxose, acetyl-digitoxose, and glucose
Diglanide C	C ₄₈ H ₇₄ O ₂₀	<i>Digitalis lanata</i> (L)	Digoxigenin	2 Digitoxose, acetyl-digitoxose, and glucose

TABLE VI—Continued

Glycoside	Formula	Plant Sources †	Hydrolytic Products	
			Genin	Sugars
Asclepiadaceae				
Periplocynarin.....	C ₃₀ H ₄₈ O ₄	Periploca graeca (W, B)	Periplogenin	Cymarose
Uzarin.....	C ₃₄ H ₅₄ O ₁₄	Uzara Tree	Anhydrouzarinigenin	2 Glucose
Periplocin.....	C ₃₄ H ₅₆ O ₁₂	Periploca graeca (B) Ghomphocarpus	Periplogenin	Cymarose and glucose
Moraceae				
α-Antiarin.....	C ₂₉ H ₄₈ O ₁₁	Antiaris toricaria (1a)	Antiarigenin	Antiarose
β-Antiarin.....	C ₂₉ H ₄₇ O ₁₁	Antiaris toricaria (1a)	Anhydroantiarigenin	Rhamnose
Liliaceae				
Convallatoxin.....	C ₂₉ H ₄₈ O ₁₀	Convallaria majalis (F, L)	Convallatoxinigenin	Rhamnose
Proscillaridin A.....	C ₃₀ H ₄₈ O ₈	Scilla maritima	Scillaridin A	Rhamnose
Scillaren A.....	C ₃₆ H ₅₂ O ₁₂	Scilla maritima	Scillaridin A	Rhamnose and glucose

* Compiled from reviews by Jacobs, Elderfield, and Tschesche, reference 269; and from Neumann, *Ber.*, **70**, 1547 (1937).
† Abbreviations: B = bark, F = flowers, L = leaves, La = latex, R = root, S = seeds, W = wood.

carboxyl group of the side chain is lactonized with the enolized form of the aldehyde group. Like other members of the cyclopentanoperhydrophenanthrene group, the genins are hydroxylated at C₃ and have an additional hydroxyl group at C₁₄. The spatial configuration of the ring nucleus is not the same in all the aglucons, but in most of them the relationship of rings A/B is probably *cis*. That of the other rings cannot be determined with certainty by the present methods, but x-ray and other physical measurements (p. 1712) show that the genins are comparable to the sterols and bile acids.²⁷³ Scillaridin A differs from the other



I Ring system of the cardiac aglucons
Rings A, B: *cis* or *trans*, generally *cis*.
C₃—OH: *trans* or *cis*, generally *trans*.]

aglucons in that the lactone ring at C₁₇ contains five carbon atoms and there is apparently no hydroxyl group at C₃. The structure of this aglucon will be discussed separately.

The principal aglucons are listed in Table VII. They differ from one another in the number of hydroxyl groups and in the stereochemistry of the ring system. The structural characteristics of the aglucons given in this table are subject to revision, but in most instances the proof is good if not rigid.

The development of the structural chemistry of these compounds parallels that of the other members of the steroid group.* The presence of a lactone side chain as a common feature was noted early in the course of the investigation.²⁷⁴ Through the work of Jacobs and collaborators, working largely with strophanthidin, this ring was char-

²⁷³ Bernal and Crowfoot, *J. Soc. Chem. Ind.*, **53**, 953 (1934). The present ring system of the cardiac aglucons was suggested by Kon, *ibid.*, **53**, 593 (1934), and these measurements were used by him, *ibid.*, **53**, 956 (1934), to confirm his previous suggestion.

* The earlier work is reviewed by Schmiedeberg, *Arch. exp. Path. Pharmacol.*, **16**, 162 (1883). Subsequent to this Kiliani, in a series of papers published in the *Berichte* (1890–1931) and *Arch. Pharm.* (1895–1913), studied the isolation of the pure glycosides. Modern constitutional investigation began in 1915 with the work of Windaus, reference 279.

²⁷⁴ Feist, *Ber.*, **31**, 534 (1898).

TABLE VII
THE CARDIAC AGLUCONS *

Aglucon	Position of OH Groups	Variation of Formula I			Formula	In-soluble Digi-tonide	M. P., ° C.	[α] _D ²⁰ (Alcohol)	Sources
		C ₇ -OH	R	Rings A B					
<i>Dihydroxygenins</i>									
Ligitoxigenin.....	3, 14	<i>trans</i>		<i>cis</i>	CH ₃	—	253	+19.1	Digitoxin
Thevetigenin.....	3, 14	<i>cis</i>		<i>cis</i>	CH ₃	+		Thevetin
Uzargenin.....	3, 14	<i>cis</i>		<i>trans</i>	CH ₃	+		Uzarin
<i>Trihydroxygenins</i>									
Digoxigenin.....	3, 11, 14	<i>trans</i>		<i>cis</i>	CH ₃	—	222	+25.8	Digoxin
Gitoxigenin.....	3, 14, 16	<i>trans</i>		<i>cis</i>	CH ₃	—	135-137 (235)	+38.5	Gitoxin
Periplogenin.....	3, 5, 14	<i>trans</i>		<i>cis</i>	CH ₃	—	135-140	+31.5	Periplocin, periplocymarin
Sarmentogenin.....	3, 11, 14	<i>trans</i>		<i>cis</i>	CH ₃	—	168-169	+21.5	Sarmentocymarin
Strophanthidin.....	3, 5, 14	<i>trans</i>		<i>cis</i>	CHO	—	265-266	+44 (MeOH)	k-Strophanthin-β, -cymarin
<i>Genins of Uncertain Structure</i>									
Convallatoxigenin †	3, 5, 8, 14	?		?	CH ₃	—	Convallatoxin
Antiarigenin.....	?, ?, ?		?	CHO (?)	—		α- and β-Antiarin
Ouabagenin.....	3, ?, ?, 14	<i>trans</i>		?	CH ₂ OH (?)	—		Ouabain
Scillaridin A §	14	CH ₃	—	211 212	Scillaren A

* Structural data from Tschesche²⁶⁹; other data from Elderfield.²⁶⁹

† Probable structures.

‡ Contains a double bond at C₉:C₁₁ (?).

§ Lactone ring contains extra carbon atom. See structure XLVIII (p. 1338)

|| Isolated as anhydrogenins.

acterized as an enolized lactone of a γ -aldehydo acid. The oxygen atoms not accounted for in the lactone ring are present as secondary and tertiary hydroxyl groups and, occasionally, in an oxygenated methyl group at C₁₀. The ring system to which these groupings are attached resisted elucidation for some years, although the opinion had long been held that it was closely related to that of the sterols and bile acids. The problem was originally attacked by the study of the products of oxidative degradation, but again it was selenium dehydrogenation of monoanhydrouzarigenin²⁷⁵ and of strophanthidin²⁷⁶ to Diels' hydrocarbon that furnished the essential clue. With this evidence available, the nuclear structure was soon more definitely characterized, and at the same time the position of attachment of the lactone ring was determined.

The precise structural characterization of the ring nucleus and the assignment of the lactone ring to C₁₇ have come from the study of the two aglucons uzarigenin (II), and digitoxigenin. Tschesche,²⁷⁷ working with monoanhydrouzarigenin, converted it by hydrogenation, cold chromic acid oxidation (C₃—OH to carbonyl), and Clemmensen reduction to a mixture of two stereoisomeric lactols (III) (stereoisomeric about C₂₀). Vigorous oxidation of these yielded a mixture of stereoisomeric dicarboxylic acids (IV), both of which gave etioallocholanolic acid (V) when degraded by the method of Barbier-Wieland. A similar degradation carried out on γ -digitoxanol diacid (p. 1335) from digitoxigenin by Jacobs and Elderfield²⁷⁸ led to the formation of etiocholanolic acid. Both of these degradations show that the lactone side chain is attached at C₁₇, but neither of them gives complete information about the spatial configuration of the nucleus. Since all the cardiac aglucons other than uzarigenin can be correlated with digitoxigenin, it would appear that they have the spatial configuration of etiocholanolic acid. This is not necessarily true, however, for in both degradations catalytic hydrogenation of a double bond at C₁₄ : C₁₅ (or C₈ : C₁₄) is necessary, and in the hydrogenation a different spatial configuration from that of the native aglucons may result. Thus these degradations furnish information only on the spatial relations of rings A/B.

The Lactone Side Chain. The degradation of monoanhydrouzarigenin to etioallocholanolic acid also shows that there are four carbon atoms

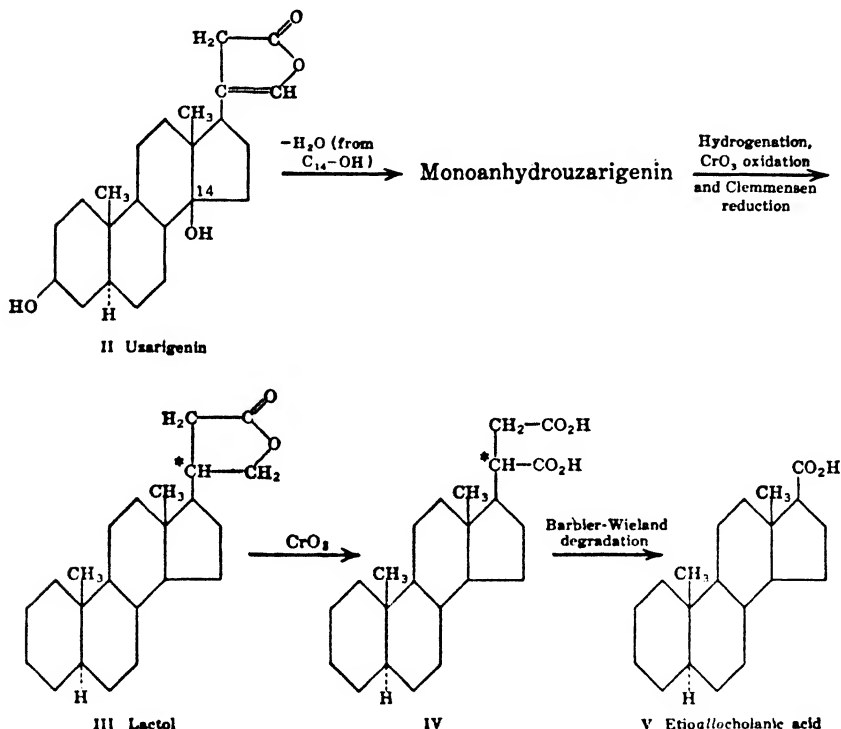
²⁷⁵ Tschesche and Knick, *Z. physiol. Chem.*, **222**, 58 (1933).

²⁷⁶ Elderfield and Jacobs, *Science*, **79**, 279 (1934); *J. Biol. Chem.*, **107**, 143 (1934); Cf. Jacobs and Fleck, *ibid.*, **97**, 57 (1932).

²⁷⁷ Tschesche, *Angew. Chem.*, **47**, 729 (1934); *Z. physiol. Chem.*, **229**, 219 (1934); *Ber.*, **68**, 7 (1935); Tschesche and Bohle, *Ber.*, **68**, 2252 (1935).

²⁷⁸ Jacobs and Elderfield, *Science*, **80**, 434, 533 (1934); *J. Biol. Chem.*, **108**, 497 (1935); this last paper is very important since it correlates Jacobs' earlier work with the newer concepts.

in the lactone side chain, though this was known from other reactions prior to this degradation. The lactone ring of the glycosides and the aglucons is readily opened by alkali at moderately elevated temperatures. Indeed, a lactone titration at an elevated temperature is one of the most satisfactory ways of determining the molecular weight of both the glycosides and the free genins.²⁷⁹ One of the α -hydrogens of



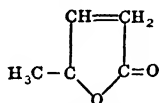
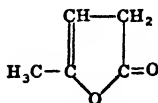
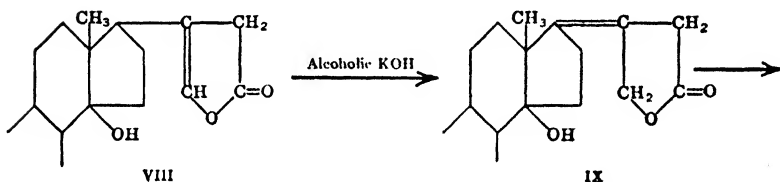
the unsaturated lactone ring is active, but, on hydrogenation to the dihydrogenins, this active hydrogen is no longer detectable.

Pyridine solutions either of the glycosides or of the genins, when treated with an alkaline solution of sodium nitroprusside, give a characteristic red color (Legal's test), but the dihydrogenins do not give this reaction. Jacobs²⁸⁰ in his earlier work was led to believe that this color reaction was due to the unsaturated lactone ring and could be interpreted to show its structure. To establish this, he examined the behavior of the $\Delta^{\alpha,\beta}$ - and $\Delta^{\beta,\gamma}$ -angelica lactones toward nitroprusside. The latter compound (VII) was found to give the same color reaction

²⁷⁹ Windaus and Hermanns, *Ber.*, **48**, 991 (1915).

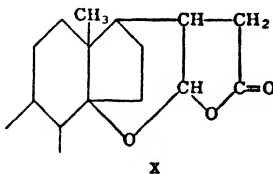
²⁸⁰ Jacobs, Hoffmann, and Gustus, *J. Biol. Chem.*, **70**, 1 (1926).

as the genins, while the $\Delta^{\alpha,\beta}$ -lactone (VI) at first gave a very weak color, which, on standing, grew stronger until finally it was comparable with that of the $\Delta^{\beta,\gamma}$ -lactone. Probably a rearrangement of the double bond is responsible for the final production of color. Similarly, toward silver solutions there is a parallel in behavior. The $\Delta^{\beta,\gamma}$ -lactones reduce ammoniacal silver solutions, the aglucons are weakly reducing, and the $\Delta^{\alpha,\beta}$ -lactone does not affect this reagent even on long standing. From these facts Jacobs concluded that the structure of the lactone side chain is that shown in formula I, and this conclusion has been confirmed by several degradations carried out since then.

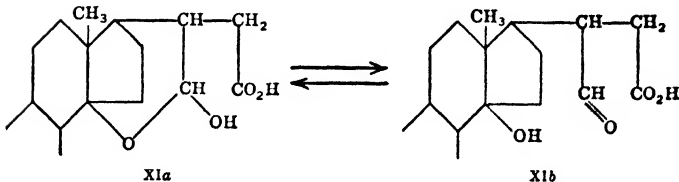
VI $\Delta^{\alpha,\beta}$ -Angelica lactoneVII. $\Delta^{\beta,\gamma}$ -Angelica lactone

VIII

IX



X



XIa

XIb

The aglucons, when treated with alcoholic alkali, undergo a characteristic rearrangement to saturated isogenins that no longer give the typical color reactions with nitroprusside. Saponification is not essen-

tial to the change, although it is possible that it may occur. Jacobs²⁸¹ has offered the following mechanism for the rearrangement; As the result of the action of alkali, the double bond at C₂₀ : C₂₁ shifts to C₁₇ : C₂₀, thus bringing the lactone group in a *cis* position with respect to an hydroxyl group (generally C₁₄—OH) γ or δ to the lactone aldehyde group. An oxide ring is then formed between the lactone ring and the hydroxyl, and, at the same time, the double bond is saturated through a rearrangement of hydrogen atoms. The change involving a C₁₄—OH group is represented in the partial structures VIII–X. On saponification of the isogenins, an equilibrium mixture of lactol (XIa) and open (XIb) forms is produced.²⁸²

The saturated dihydrogenins are not isomerized by alcoholic alkali, nor are the anhydrogenins that are produced by the action of alcoholic hydrochloric acid generally susceptible to this rearrangement. In the former case there is no labile double bond, and in the latter the C₁₄—OH has been removed as water by the action of the reagent. The correct interpretation of this characteristic reaction has been of great value in determining the structure of the cardiac substances.

The Structure of Strophanthidin

The genin strophanthidin has been studied more than any other aglucon, as it is relatively easy to obtain and is not in demand for pharmaceutical preparations. The chemistry of this compound gives a good picture of the type of problem that is met in the study of the aglucons, but the awkward terminology that has grown up for strophanthidin and its derivatives (and those of the other genins) is a real handicap in understanding the various transformations.

Isolation. Strophanthidin (XII) is obtained from the glycosides present in several varieties of *Strophanthus*. From *S. kombé*, for example, a mixture of cymarín and k-strophanthin- β , together with uncharacterized amorphous glycosides, is obtained. Cymarín may be separated from the mixture by dissolving it out with chloroform,²⁸³ and on hydrolysis it yields the aglucon and the sugar cymarose, C₇H₁₄O₄.²⁸³ k-Strophanthin- β , in turn, gives strophanthidin and a disaccharide, C₁₃H₂₄O₉, composed of cymarose and glucose; glycosidic union with the genin is through cymarose.²⁸⁴ Aside from the unsaturated lactone ring, strophanthidin possesses a free aldehyde group at C₁₀, tertiary

²⁸¹ Jacobs and Collins, *ibid.*, **61**, 387 (1924). Cf. Feist, *Ber.*, **33**, 2063, 2069, 2091 (1900), and Windaus, reference 279.

²⁸² Jacobs and Gustus, *J. Biol. Chem.*, **74**, 811 (1927).

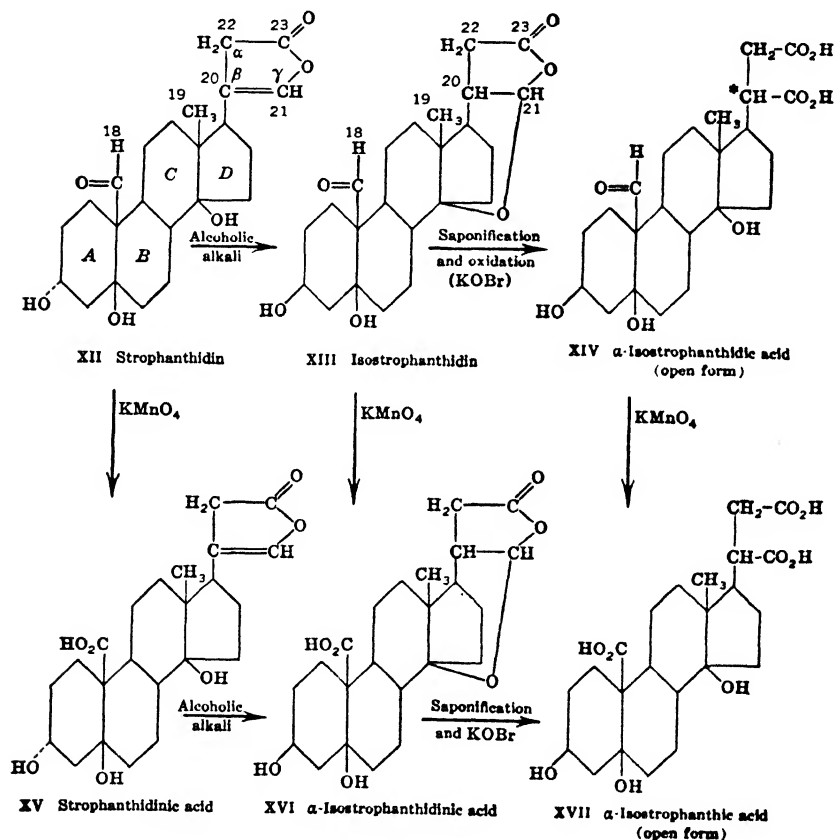
²⁸³ Jacobs and Hoffmann, *ibid.*, **67**, 609 (1926).

²⁸⁴ Jacobs and Hoffmann, *ibid.*, **69**, 153 (1926).

hydroxyl groups situated at C₅ and C₁₄, and a secondary hydroxyl group at C₃.

The Lactone Ring. On titration the unsaturated lactone ring of strophanthidin consumes one equivalent of alkali, and on catalytic reduction it absorbs one mole of hydrogen to form a dihydrogenin.²⁸⁵ That the lactone ring contains four carbon atoms is shown by the oxidation of trianhydrostrophanthidin, which is discussed later.

The C₁₄—OH Group. In the change strophanthidin (XII) → isostrophanthidin (XIII) the hydroxyl group involved must be tertiary,

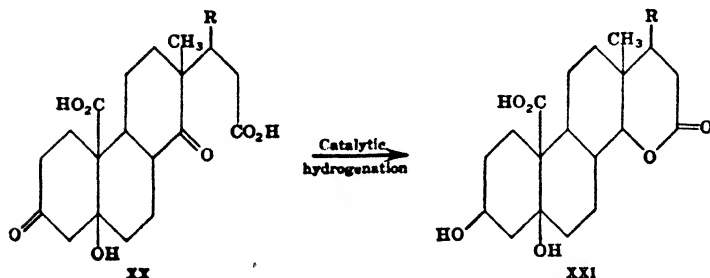
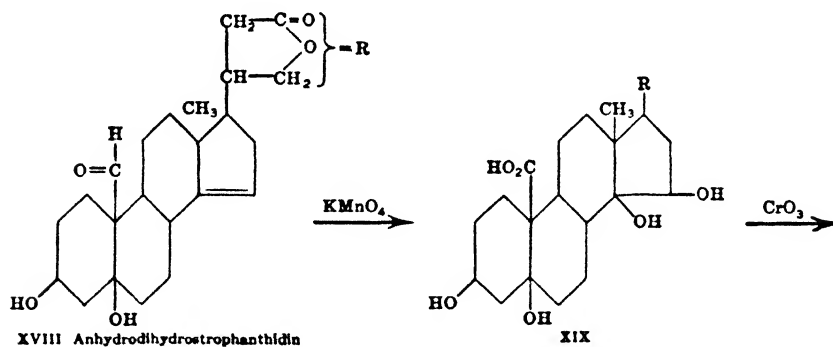


since the monoanhydrostrophanthidin produced by the action of alcoholic hydrochloric acid cannot be isomerized. To meet the requirement of being tertiary as well as γ or δ with respect to the aldehyde group of the side chain, C₁₄ is the only position that can be considered for this hydroxyl group.

²⁸⁵ Jacobs and Heidelberg, *ibid.*, **54**, 253 (1922).

Some of the products that can be formed from strophanthidin and isostrophanthidin by the proper choice of oxidizing agent are of importance in subsequent arguments and further illustrate the isomerization under consideration. Hypobromite acts selectively on the aldehyde grouping of the lactone ring after saponification, and permanganate will oxidize the C_{10} —CHO group without affecting the side chain if it has not been saponified. By the judicious application of these oxidizing agents all the possible carboxylic acids from strophanthidin and isostrophanthidin have been realized.^{282, 286} The products of the transformations are shown in structures XII–XVII. Of these α -isostrophanthidic (XIV) and α -isostrophanthic (XVII) acids are of greatest importance for subsequent consideration. It should be noted that isostrophanthidin and all the products derived from it are capable of existing in two forms due to the new center of asymmetry produced at C_{20} as a result of the isomerization.

Further evidence for the attachment of an hydroxyl group at C_{14}



comes from the study of dihydrostrophanthidin.²⁸⁷ By the action of alcoholic hydrogen chloride the dihydrogenin is converted to an anhydride (XVIII) which is unsaturated at C₁₄:C₁₅. Treatment of this anhydride with potassium permanganate produces a glycol (XIX), and, at the same time, the C₁₀—CHO is oxidized to carboxyl. Further oxidation with chromic acid opens ring D and converts the C₃—OH to carbonyl (structure XX). On catalytic hydrogenation the carbonyl groups at C₃ and C₁₄ are both reduced to hydroxyl groups and the product is isolated as the lactol of structure XXI. The spontaneous formation of the lactone indicates a γ - or δ -lactone. The transformation XVIII—XXI is explicable only on the basis of an hydroxyl at C₁₄ in the parent compound, dihydrostrophanthidin.

The C₁₀—CHO Group. Reduction of the aldehyde group of α -isostrophanthidic acid (XIV) to methyl by the Wolff-Kishner method gives isoperiplogenic acid, which, in turn, can be prepared from digitoxigenin.²⁸⁸ Since the latter, through its correlation with etiocholanolic acid, is known to have methyl groups at C₁₀ and C₁₃, the aldehyde group of strophanthidin must be attached at one of these positions, but C₁₃ is eliminated by the following considerations: In the production of monoanhydrostrophanthidin by the action of alcoholic hydrochloric acid, the anhydrogenin is isolated as a cyclo-half-acetal. This compound does not possess the properties of an aldehyde or of a secondary alcohol, but on hydrolysis these functions are regenerated. Since the secondary hydroxyl group can be shown to be attached at C₃ by other reactions, and the aldehyde group must be in a γ or δ relationship to this hydroxyl to form a cyclo-acetal, C₁₀ is the only possible position of attachment.

The steric relations involved in the formation of the acetal are brought out by a similar lactonization that takes place with the β -isostrophanthidin derivatives.²⁸⁹ When α -isostrophanthidic acid (XIV) is boiled with alkali, it rearranges to β -isostrophanthidic acid in which the aldehyde group exists both free and as the lactal in combination with the secondary hydroxyl group. On oxidation with permanganate, the aldehyde group at C₁₀ is converted to carboxyl and this acid also readily lactonizes. Since the compounds of the α -isostrophanthidin series do not lactonize in this way, a rearrangement must occur in the alkaline treatment to bring the aldehyde group and the secondary hydroxyl into a *cis* configuration with respect to each other. Tschesche and Bohle²⁹⁰ have suggested for the isomerization to the β -series that

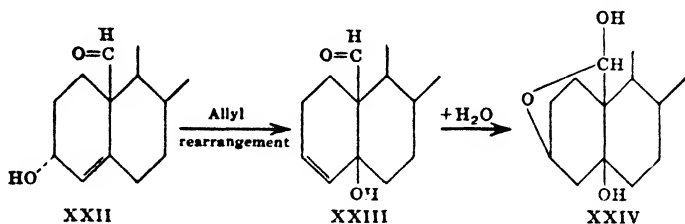
²⁸⁷ Jacobs and Elderfield, *ibid.*, **113**, 611 (1936).

²⁸⁸ Jacobs, Elderfield, Grave, and Wignall, *ibid.*, **91**, 617 (1931); Jacobs and Elderfield, *ibid.*, **91**, 625 (1931).

²⁸⁹ Jacobs and Gustus, *ibid.*, **74**, 829 (1927).

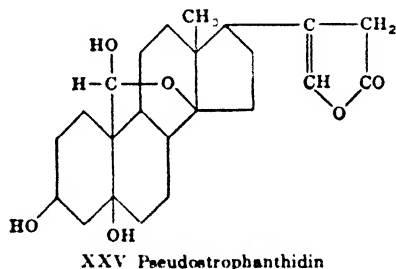
²⁹⁰ Tschesche and Bohle, *Ber.*, **69**, 2443 (1936).

the treatment with alkali first splits off the C_5-OH , that the resulting unsaturated compound (XXII) then undergoes allyl rearrangement to XXIII, and that water finally adds to the double bond to give an hydroxyl group at C_3 *cis* to the $C_{10}-CHO$ (structure XXIV). Possibly the same kind of transformation occurs in the formation of the ethyl



acetal of monoanhydrostrophanthidin.²⁹¹ It may be recalled in this connection that in the Walden inversion about C_3 in other members of the group a satisfactory mechanism has not been developed.

By the action of concentrated hydrochloric acid the $C_{10}-CHO$ group may be brought into reaction with the $C_{14}-OH$ to form the



so-called pseudostrophanthidin, to which formula XXV has been assigned.²⁹² The structure appears to be satisfactory, since pseudostrophanthidin cannot be isomerized, contains a secondary hydroxyl group, and gives the reactions to be described later for the C_5-OH group. The formation of such a compound, however, may involve a steric rearrangement, since spatially strophanthidin appears to resemble epicoprosterol and the $C_{10}-CHO$ and the $C_{14}-OH$ are presumably *trans* to each other (*cf.* coprostane model, p. 1252).

The C_3-OH Group. The first evidence that an hydroxyl group is attached at C_3 in the cardiac aglucons, and therefore in strophanthidin, was obtained from the study of a derivative of dihydrogitoxigenin (see below) in which all the tertiary hydroxyl groups had been replaced with

²⁹¹ *Cf.* Elderfield, *Chem. Rev.*, **17**, 229 (1935).

²⁹² Jacobs and Collins, *J. Biol. Chem.*, **63**, 123 (1925).

hydrogen. Vigorous oxidation of this genin derivative cleaved the ring bearing a secondary hydroxyl group and gave a dibasic acid. When the dibasic acid was subjected to thermal decomposition, a pyro ketone was obtained. The reaction was carried out by Windaus²⁹³ when the structure of cholesterol was still unknown, and at that time the formation of a pyro ketone could not be correctly interpreted. Windaus recognized that the parallel behavior of this diacid and the one formed by similar treatment of cholesterol indicated a correspondence of structure.

The definite placement of a secondary hydroxyl group in strophanthidin at C₃ was later made by a series of reactions analogous to those used in locating the C₃—OH of cholesterol. With α -isostrophanthic dimethyl ester (XXVI) as a starting point, cold chromic acid oxidation gives the corresponding ketone, α -isostrophanthonic dimethyl ester (XXVII). The latter readily loses water to give the unsaturated ketone (XXVIII), which, when treated with ozone, is cleaved in ring A to form undephanthotriacid dimethyl ester²⁹⁴ (XXIX). On treatment with weak alkali, this keto acid (XXIX) suffers β -ketone decomposition and is converted to duodephanthondiacid (XXX), and by the action of acetic anhydride-acetyl chloride, the diacid is transformed to an unsaturated lactone²⁹⁵ (XXXI), which may be catalytically reduced to the saturated dephanthanic acid (XXXII). Barbier-Wieland degradation of dephanthanic acid converts it with the loss of four carbon atoms to dephanthic acid;²⁹⁶ three of these carbon atoms come from the C₁₇ side chain, the fourth is formed by shortening the fragment of ring A. These reactions show that a sequence, —CH₂—CHOH—CH₂—, is present in one of the rings and terminates at a tertiary carbon. This sequence can be accommodated only in ring A, and the requirement of termination in a tertiary carbon atom places the hydroxyl group definitely at C₃.

Since strophanthidin does not give an insoluble digitonide, the C₃—OH group is probably *trans* to the C₁₀—CHO.²⁹⁷ The evidence is inconclusive, however, since cholestanetriol (3,5,6-trihydroxy), in which the C₃—OH is presumably *cis* to the C₁₀—CH₃, fails to give an insoluble digitonide (p. 1259).

The C₅—OH Group. As mentioned above, the dehydration of α -isostrophanthonic acid (XXVII) proceeds with great ease. Since such dehydrations are typical of β -hydroxy ketones, a tertiary hydroxyl

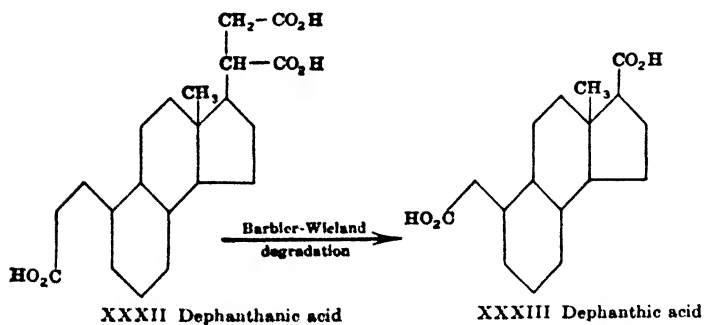
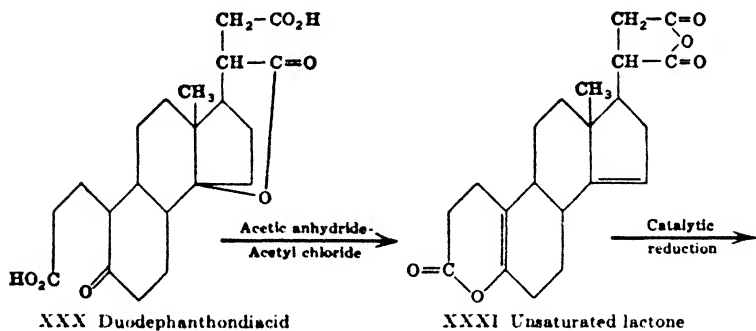
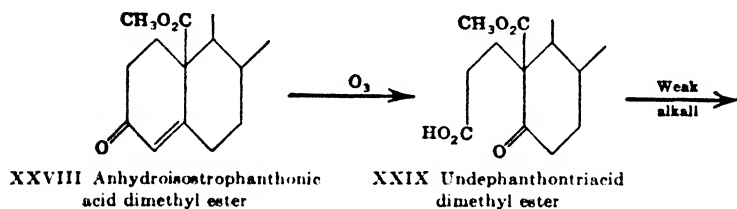
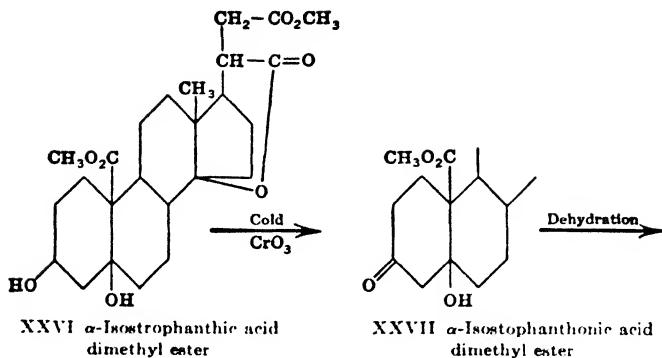
²⁹³ Windaus, Westphal, and Stein, *Ber.*, **61**, 1847 (1928).

²⁹⁴ Jacobs and Gustus, *J. Biol. Chem.*, **79**, 539 (1928).

²⁹⁵ Jacobs and Gustus, *ibid.*, **92**, 323 (1931).

²⁹⁶ Jacobs and Elderfield, *ibid.*, **102**, 237 (1933).

²⁹⁷ Tschesche and Bohle, *Ber.*, **68**, 2252 (1935).



group is placed at C₅, for only at this position can an hydroxyl group be both β to C₃ and tertiary. The C₅—OH group is probably *cis* to the C₁₀—CHO. This is shown by the following evidence from the work of Jacobs and Elderfield:²⁹⁸ When dihydrostrophanthidin is treated with hydrogen cyanide, the C₁₀—CHO is converted *via* a cyanohydrin to two isomeric α -hydroxy acids. Both these acids readily form lactones (so-called homolactones) through interaction with the C₅—OH. The involvement of this hydroxyl group in the formation of homolactones is shown by the fact that anhydrostrophanthidin gives similar reactions, and that the lactones can be converted to ketones (OH at C₃) or formed after protection of the C₃—OH by benzylation. Tschesche²⁹⁹ has pointed out that this ease of lactone formation indicates a *cis* relationship between the C₅—OH and the C₁₀—CHO. (Cf. Alder-Stein rule, p. 1261.)

The Anhydrostrophanthidins. Dehydration of monoanhydrostrophanthidin to dianhydrostrophanthidin is effected by heating the acetal of anhydrostrophanthidin with alcoholic hydrogen chloride. The product obtained is the ethyl hemiacetal of dianhydrostrophanthidin (XXXIV), formed by the loss of the C₅—OH. When dianhydrostrophanthidin is treated with concentrated aqueous hydrochloric acid, another molecule of water is lost with the formation of trianhydrostrophanthidin²⁹² (XXXV). With the exception of the double bond of the lactone ring, the trianhydrogenin shows none of the properties of an unsaturated compound, and when oxidized with fuming nitric acid, yields 1,2,3,4-benzenetetracarboxylic acid.²⁹⁹ The production of this tetracarboxylic acid is explicable if, in the formation of trianhydrostrophanthidin, the C₁₀—CHO wanders to C₁ with a simultaneous shift of bonds to produce aromatization of ring B. Fieser³⁰⁰ has suggested that the reaction may be explained by an enlargement of ring A to a seven-membered ring, rather than a shift of the aldehyde group. There is some question as to whether this change takes place in the production of trianhydrostrophanthidin or whether it has already occurred in the formation of the dianhydrogenin.³⁰¹ The lactone ring of strophanthidin is, in general, quite resistant to oxidizing agents. In trianhydrostrophanthidin, however, the ring is easily oxidized away to give the acid of the probable structure shown in formula XXXVI.³⁰²

In the previous discussion the nuclear double bond in monoanhydrostrophanthidin has been assigned to C₁₄ : C₁₅. This is apparently true

²⁹⁸ Jacobs and Elderfield, *J. Biol. Chem.*, **113**, 625 (1936).

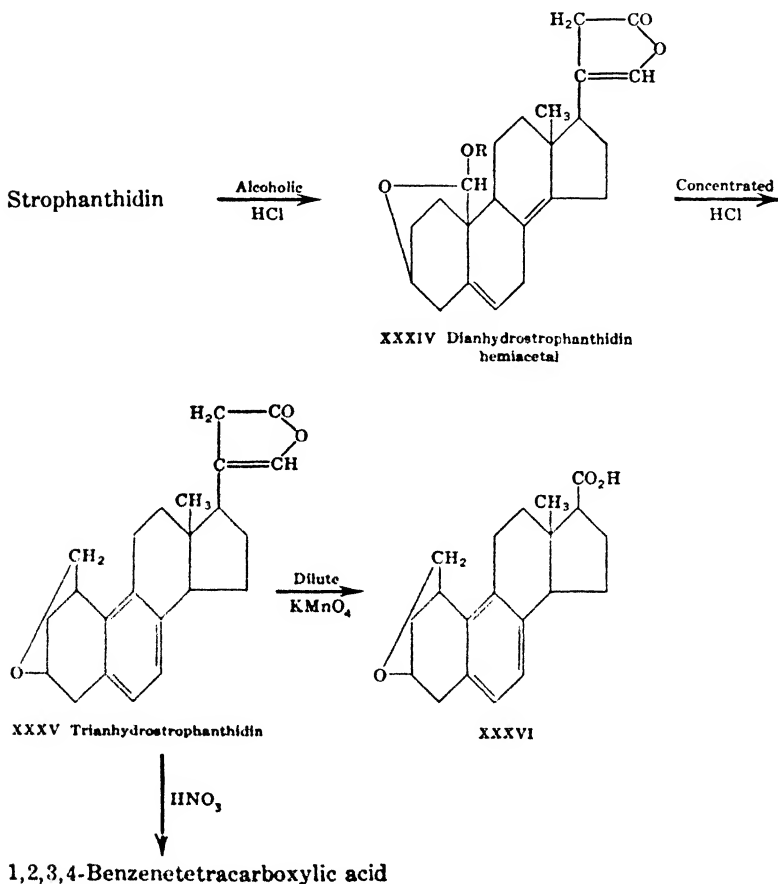
²⁹⁹ Tschesche and Knick, *Z. physiol. Chem.*, **239**, 233 (1934).

³⁰⁰ Fieser, Monograph p. 274.

³⁰¹ Elderfield, *Chem. Rev.*, **17**, 225 (1935).

³⁰² Jacobs and Gustus, *J. Biol. Chem.*, **74**, 805 (1927).

for alkaline media, but in certain reactions in acid or neutral media there is evidence that the double bond shifts to $C_8 : C_{14}$ (cf. α -stenols and apocholic acid). In dianhydrostrophanthidin there is another anomaly. When the lactone side chain of monoanhydrostrophanthidin is saponified, the aldehyde acid obtained does not relactonize.³⁰³ Apparently



this is due to a rearrangement of the lactone double bond into ring D to form a conjugated system $C_{14} : C_{15} \cdot C_{16} : C_{17}$. The double bonds of monoanhydro- and dianhydrostrophanthidin can be preferentially hydrogenated; as in certain of the sterols the nuclear double bonds are hydrogenated more easily than the double bond of the side chain.²⁹²

The stereochemistry of these anhydrostrophanthidins and certain

³⁰³ Jacobs and Elderfield, *ibid.*, **108**, 693 (1935).

other unexplained products such as the γ - and δ -strophanthidin series²⁸⁹ is a problem of the future.

Interrelationship of the Aglucons

The aglucons have been correlated through conversion to mutually common compounds. In this manner they have been shown to have the same ring system and an hydroxyl group at C₃. Since digitoxigenin, gitoxigenin, strophanthidin, and anhydrosarmentogenin do not give insoluble digitonides, the C₃—OH is probably *trans* to the C₁₀—R in these genins and those that can be simply related to them. All the aglucons or their glycosides give a positive Legal's test, isomerize when treated with alcoholic alkali, and are dehydrated by alcoholic hydrochloric acid. Thus the unsaturated lactone ring and an hydroxyl group at C₁₄ are common characteristics. The number and position of other hydroxyl groups have been determined by special methods. Brief summaries of the work leading to the present structures are given below.

Periplogenin. As cited above, isoperiplogenic acid may be formed by reduction of the C₁₀—CHO of α -isostrophanthidic acid (XIV) to C₁₀—CH₃. Periplogenin is, therefore, a desoxostrophanthidin.

Digitoxigenin.³⁰⁴ Oxidation with chromic acid of the methyl ester of isoperiplogenic acid converts it to a ketonic ester (carbonyl at C₃), which readily loses water (C₅—OH) to form an anhydro ketone. On hydrogenation of the anhydro compound a mixture of stereoisomeric dihydro compounds is obtained; one of these (isodigitoxigenic ester) is identical with an ester formed from digitoxigenin. The two hydroxyl groups of digitoxigenin must be attached at C₃ and C₁₄. Digitoxigenin does not form an insoluble digitonide and the C₃—OH must be *trans* to the C₁₀—CH₃. The degradation of digitoxigenin to etiocholanolic acid via γ -digitoxanol diacid (p. 1320) shows that the relation of rings A/B is *cis*.

Thevetigenin.³⁰⁵ When thevetin is hydrolyzed with hydrochloric acid, two molecules of glucose and one of water are split off to give monoanhydroprothevetigenin, a partially desugared product which probably contains digitalose. The hydroxyl group lost in the hydrolysis is presumably attached at C₁₄, since potassium hydroxide isomerizes the glycoside but not the hydrolytic product. After the partially desugared genin is saturated to a tetrahydro compound, it may be completely hydrolyzed. The resulting tetrahydroanhydrothevetigenin is converted by careful oxidation with chromic acid into tetrahydroanhydrodigitoxigenone, thus placing the second hydroxyl group at C₃. The

³⁰⁴ Jacobs and Elderfield, *ibid.*, **92**, 313 (1931).

³⁰⁵ Elderfield, *ibid.*, **115**, 247 (1936); Tschesche, *Ber.*, **69**, 2368 (1936).

C₃—OH must be *cis* to the C₁₀—CH₃ group, since anhydrothevetigenin forms an insoluble digitonide.

Uzarigenin.^{275, 277} The aglucon uzarigenin has been isolated only in the form of its monoanhydrogenin. The glycoside is isomerized by alkali, but the anhydrogenin is not. Thus the hydroxyl group that is lost in hydrolysis is attached at C₁₄ in the genin itself. By correlation with periplogenin a second hydroxyl group has been placed at C₃. Since the anhydrouzarigenin forms an insoluble digitonide, this C₃—OH is *cis* to the C₁₀—CH₃. The degradation of monoanhydrouzarigenin to etioallocholanolic acid (p. 1320) shows that the relationship of rings A/B is *trans*. Uzarigenin appears to be the only aglucon in which these rings have this relationship.

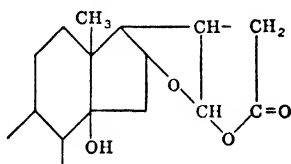
Digoxigenin.³⁰⁶ Although the aglucon digoxigenin contains two secondary and one tertiary hydroxyl groups, the diketone resulting from oxidation of the genin gives mono derivatives with ketone reagents. When the aglucon is dehydrated, two anhydrodigoxigenins are produced (double bond at C₁₄ : C₁₅ or C₈ : C₁₄) and catalytic reduction of the principal dehydration product converts it to a tetrahydroanhydrogenin from which a diketone is obtained by cold chromic acid oxidation. Clemmensen reduction of the diketone converts it to a saturated lactone which is identical with a lactone prepared from digitoxigenin in a similar way. Therefore the ring system of digoxigenin is the same as that of digitoxigenin. Oxidation of the diketone converts it to a ketodicarboxylic acid which, on pyrolysis, yields a pyro ketone. This behavior indicates an hydroxyl group attached to ring A, probably at C₃. The other secondary hydroxyl group may be placed at C₁₁ by eliminating the rest of the positions through the following considerations: The diketone from the cold chromic acid oxidation is rearranged when treated with alkali, but does not form a pyridazine with hydrazine. The ability to rearrange in the presence of alkali shows that the carbonyl group under discussion is adjacent to a hydrogen attached to the bridge head of two rings, and the inability of the diketone to form a pyridazine indicates that the carbonyl is not attached at C₆. The anhydro diketone from digoxigenin does not absorb ultra-violet light, and because of this the double bond formed by loss of the C₁₄—OH cannot be in conjugation with a carbonyl group. Attachment of the hydroxyl at C₇, C₁₅, or C₁₆ is therefore eliminated. Finally, attachment at C₁₂ is not possible, because the saturated diketone rearranges when treated with alkali.

Gitoxigenin. The aglucon gitoxigenin (XLI) possesses one tertiary (C₁₄) and two secondary hydroxyl groups. One of these secondary

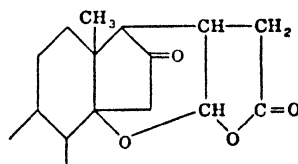
³⁰⁶ Smith, *J. Chem. Soc.*, 508 (1930); 23 (1931); 1050, 1305 (1935); 354 (1936); Tschesche and Bohle, *Ber.*, **69**, 793 (1936).

groups is attached at C₃, and the other has been placed at C₁₆. This second hydroxyl group enters into reactions with the unsaturated lactone side chain, so that alkaline isomerization of this genin is somewhat different from the normal.³⁰⁷ The isogenin (XXXVII) formed by treatment with alkali is unusually stable, and the lactone of the oxidation product, isogitoxigenic acid, is relatively resistant to hydrolysis. If gitoxigenin is oxidized with chromic acid to diketogitoxigenin, the product does not give a positive Legal reaction. This is probably due to a spontaneous formation of an isogenone through interaction with the C₁₄—OH. The mechanism of the reaction may be a shift of the double bond of the lactone side chain into conjugation with the carbonyl double bond at C₁₆. The probable structure of the isogenone is shown in formula XXXVIII.

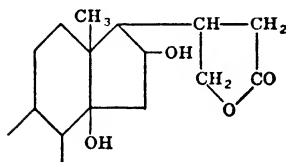
On hydrogenation gitoxigenin is converted into two isomeric α - and β -dihydrogenins.³⁰⁸ Both these dihydrogitoxigenins undergo mutarotation, probably through a rearrangement involving the lactone group. Structures XXXIX and XL represent the probable configurations of



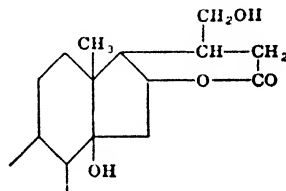
XXXVII
Isogitoxigenin



XXXVIII
Isogitoxigenone



XXXIX
 α -Dihydrogitoxigenin (dextro)



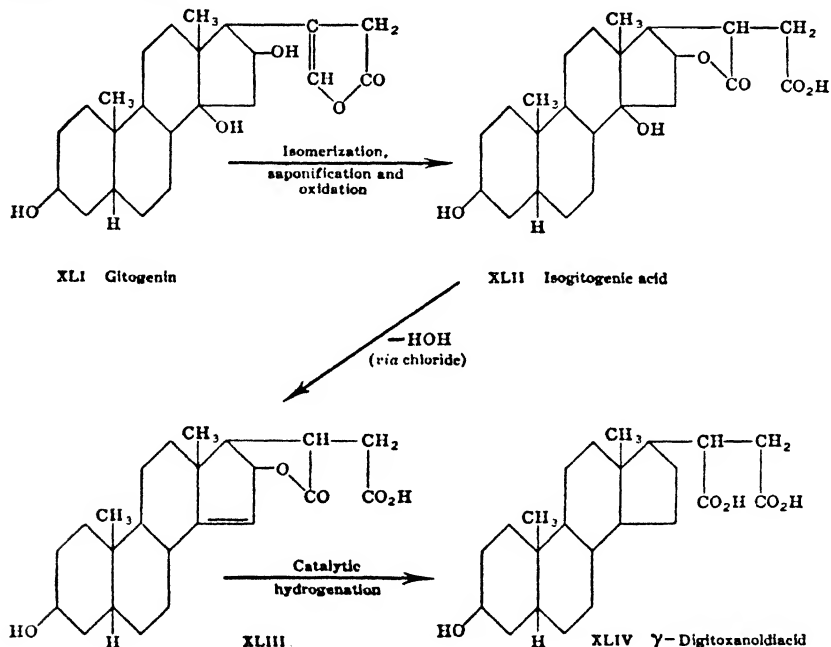
XL
 β -Dihydrogitoxigenin (levo)

these two dihydrogitoxigenins. As is evident from these structures, in the β -form lactonization has occurred on the secondary hydroxyl group at C₁₆. On oxidizing the α -dihydrogenin a dihydrogitoxigenone is obtained. This ketone is easily dehydrated, as would be expected of a compound in which the tertiary hydroxyl group is in a β -position with respect to the carbonyl group.

³⁰⁷ Jacobs and Gustus, *J. Biol. Chem.*, **79**, 553 (1928); **82**, 403 (1929); **88**, 531 (1930).

³⁰⁸ Jacobs and Elderfield, *J. Biol. Chem.*, **100**, 671 (1933). Cf. Windaus, *et al.*, reference 293.

Gitoxigenin (XLI) has been correlated with digitoxigenin by the following reactions:³⁰⁹ After isomerization the isogitoxigenin was saponified and oxidized to isogitoxigenic acid (XLII), which differs from the usual isogenic acids in that lactonization takes place with the C_{16} -OH. The C_{14} -OH was converted to a chloride, and the chloro acid transformed to an unsaturated acid (XLIII) by splitting out hydrogen chloride. On catalytic hydrogenation the lactone ring was opened, the C_{16} -OH group replaced, and the double bond saturated. The resulting acid was the digitoxanoldiacid (XLIV) previously mentioned (p. 1320).



Oleandrin is an acetyl glycoside of gitoxigenin.^{309a} Hydrolysis of oleandrin with acid gives the sugar oleandrose, $C_7H_{14}O_4$ (a methyl ether of a methyldeoxypentose) and the genin oleandrigenin, $C_{25}H_{36}O_6$. Alkaline hydrolysis converts oleandrigenin to gitoxigenin and acetic acid. In oleandrin, gitoxigenin is acetylated at C_{16} .

Sarmentogenin.³¹⁰ Of the three hydroxyl groups in sarmentogenin, two can be acetylated, but the third is tertiary. The tertiary hydroxyl

³⁰⁹ Jacobs and Gustus, *J. Biol. Chem.*, **86**, 199 (1930). Cf. Windaus and Freese, *Ber.*, **58**, 2503 (1925), and earlier papers.

^{309a} Neumann, *Ber.*, **70**, 1547 (1937); Tschesche, *Ber.*, **70**, 1554 (1937).

³¹⁰ Tschesche and Bohle, *Ber.*, **69**, 2497 (1936). Cf. Tschesche, *Ber.*, **68**, 423 (1935); Jacobs and Hoffmann, *J. Biol. Chem.*, **79**, 531 (1928).

group is probably attached at C₁₄, since isogenins are formed and the hydroxyl is easily removed. Careful oxidation of the genin gives a diketone, but only one of the carbonyl groups is reactive. The two secondary hydroxyl groups have been placed at C₃ and C₁₁ by the following reactions: Hydrolysis of the glycoside with alcoholic hydrochloric acid gives an α -anhydrosarmentogenin, which by catalytic reduction is converted into an α -tetrahydroanhydrosarmentogenin. When this tetrahydroanhydrogenin is oxidized with chromic acid, a ketodicarboxylic acid is obtained, which yields a pyro ketone on thermal decomposition. This behavior is typical for an hydroxyl at C₃. Controlled oxidation of the tetrahydroanhydrogenin converts it to a mixture of products from which a diketone can be isolated. On Clemmensen reduction the diketone is transformed to a monoketone. This is finally converted to a saturated lactone by catalytic reduction to an alcohol, dehydration, and repeated reduction. The saturated lactone agrees in properties with a product obtained by similar procedures from digoxigenin. Sarmentogenin and digoxigenin are therefore stereoisomeric, presumably in the manner of fusion of rings B and C. In digoxigenin these rings may have the normal *trans* configuration, and in sarmentogenin an abnormal *cis* arrangement.

The inertness of groupings attached at C₁₁ in either digoxigenin or sarmentogenin distinguishes this position. The saturated alcohol obtained from the monoketone of sarmentogenin mentioned above is hydroxylated at this point and the hydroxyl group is so inert that it does not react with benzoyl chloride in pyridine solution nor can chlorine be substituted for this hydroxyl by treatment with thionyl chloride or phosphorus pentachloride. The double bond of the dehydration product ($\Delta^{9,10}$ -dihydrolactone) of this alcohol cannot be hydrogenated in alcoholic media, but does take up hydrogen in glacial acetic acid.

Antiarigenin.³¹¹ Little is known of the aglucon antiarigenin. On hydrolyzing the glycoside, β -antiarin, two molecules of water are lost because of the very drastic conditions necessary for the hydrolysis; probably the hydroxyl groups removed are tertiary. There is one secondary hydroxyl group in the molecule, as shown by benzoylation of the dianhydrogenin, and a carbonyl group is indicated by the uptake of hydrogen in catalytic hydrogenation. Tschesche has suggested that antiarigenin differs from strophanthidin by an additional hydroxyl group.

Convallatoxigenin.³¹² The glycoside convallatoxigenin, from the lily-of-the-valley, contains two double bonds, one of which is reduced

³¹¹ Kiliani, *Ber.*, **43**, 3574 (1910); **46**, 667, 2179 (1913); Tschesche and Haupt, *Ber.*, **69**, 1377 (1936).

³¹² Jacobs and Bigelow, *J. Biol. Chem.*, **96**, 647 (1932); **101**, 15 (1933). Flesser and Newman, *ibid.*, **114**, 705 (1936). Tschesche, *Ber.*, **70**, 43 (1937).

with difficulty by catalytic hydrogenation. Since the glycoside gives a typical color reaction, one of these double bonds is in the lactone ring and the other presumably in the ring nucleus. On hydrolysis the glycoside loses water to give a non-crystalline monoanhydrogenin which contains three double bonds, but does not absorb light in the ultra-violet. As the glycoside can be isomerized and the anhydrogenin cannot, the hydroxyl group of the genin lost in hydrolysis was probably attached at C₁₄. The absence of absorption in the ultra-violet shows that the double bond formed in hydrolysis is not in conjugation with the original nuclear double bond. The monoanhydrogenin contains one secondary and two tertiary hydroxyl groups. The secondary group is probably located at C₃ and the two tertiary hydroxyl groups at C₅ and C₈. The nuclear double bond is presumably at C₉ : C₁₁.

Ouabagenin.³¹³ A structure for this genin cannot be given. The usual methods of hydrolysis are not satisfactory, as ouabain is made up of a genin united with rhamnose. The glycoside is isomerized when treated with alkali, thus suggesting an hydroxyl group at C₁₄. When the glycoside is subjected to acetolysis, a molecule of formaldehyde is formed, and, at the same time, one of the rings becomes benzenoid. This has been interpreted to indicate the grouping -CH₂OH at C₁₀. There is probably an hydroxyl group at C₃ and three additional hydroxyl groups at other positions

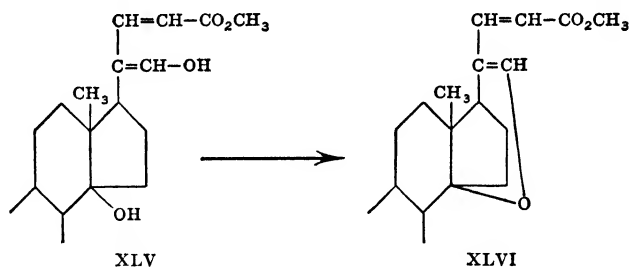
The Squill Aglucon

Two glycosides, scillaren A and B, have been obtained from the bulbs of the squill (*Scilla maritima*) by Stoll and co-workers,³¹⁴ but only the first of these has been obtained in pure form. By enzymatic hydrolysis scillaren A is split to give proscillaridin A and glucose. By means of acids, proscillaridin A may be hydrolyzed, yielding a molecule of rhamnose and, with the loss of water, the aglucon scillaridin A. Although the glycoside and the genin contain a titratable lactone grouping, neither gives Legal's test. On catalytic hydrogenation scillaren A takes up three moles of hydrogen, and scillaridin A four moles. The genin is easily dehydrated to an anhydrogenin, which, by catalytic hydrogenation, yields a mixture of acids from which *alcoholanic acid* (p. 1299) has been isolated. The formation of this acid shows quite simply that the lactone side chain of scillaridin A contains five rather than four carbon atoms.

³¹³ Karrer, *Helv. Chim. Acta*, **12**, 506 (1929); Tschesche and Haupt, *Ber.*, **69**, 459 (1936); Fieser and Newman, *J. Biol. Chem.*, **114**, 705 (1936); Tschesche and Haupt, *Ber.*, **70**, 43 (1937).

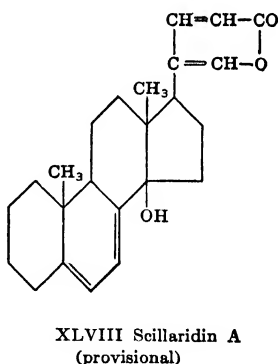
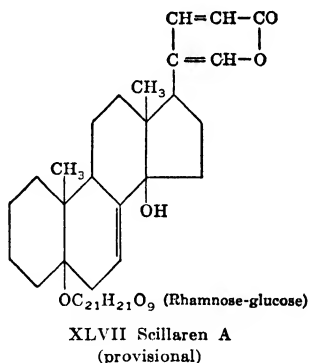
³¹⁴ Stoll and co-workers, *Z. physiol. Chem.*, **223**, 24 (1933); *Helv. Chim. Acta*, **17**, 641, 1334 (1934); **18**, 82, 120, 401, 644, 1247 (1935).

When scillaridin A is treated with methanolic potassium hydroxide, the lactone ring is opened with the formation of an ester (XLV), rather



than a potassium salt. This ester readily loses water to form a derivative (XLVI) of isoscillaridin A. The anhydrogenin is likewise esterified by methanolic potassium hydroxide, but the ester from anhydroscillaridin A is stable and cannot be isomerized, nor does it give the expected reactions of an enol. Its abnormal behavior may be due to rearrangement of the C₂₀ double bond into ring D as has been noted with the anhydrostrophanthidins.

Scillaridin A absorbs light in the ultra-violet at 290–300 mμ. This has been interpreted by Stoll to indicate a conjugated system in ring B comparable to that of ergosterol, but the absorption is not that of the



dehydrosterols and is probably due exclusively to the unsaturated lactone ring (see Toad Poisons). Stoll prefers the dehydrosterol structure, however, and offers in support the positive Rosenheim test (p. 1272) that scillaridin A gives. In any event, the hydroxyl group to which the sugar residues are attached is split off during hydrolysis. Assuming that this hydroxyl is at C₅, Stoll has offered the provisional formulas XLVII for scillaren, and XLVIII for scillaridin A. It is noteworthy that according

to these formulations scillaridin A is one of the few compounds of this group in which there is no hydroxyl group at C₃.

The Toad Poisons

The venom secreted in the parotid gland of the toad is a complicated mixture consisting of several conjugated and free genins. Other substances present include adrenaline,³¹⁵ bufotenidine and related tryptamines,³¹⁶ sterols,³¹⁷ fats, etc.* The toad poisons proper are suberylarginine derivatives of the genins. The nature of the genins, or bufagins, as they are called, varies with the species, and they may be differentiated by means of a prefix indicating the toad from which they are isolated.³¹⁸ As a result of enzyme action, hydrolysis of the venom to the free genins occurs in the toad secretion, but with acid hydrolysis dehydration takes place and only anhydrogenins are obtained. The bufagins are polyhydroxy or acetylated hydroxy lactones, containing 23 or 24 carbon atoms, and are more closely allied with scillaridin A than with the other aglucons. Many of them give a positive Liebermann test, and none gives Legal's test.

Marinobufagin (or bufagin), C₂₄H₃₄O₅, m.p. 213°, from the toad *Bufo marinus* was the first genin to be isolated,³¹⁹ but bufotalin, C₂₄H₃₄O₅(CH₃CO), m.p. 148°, from the common European toad, *Bufo vulgaris*, has been studied more extensively. The parotid secretion of this toad may be collected without injury to the animal by expressing it with tweezers. The exudate is collected in a dish or absorbed in cotton

³¹⁵ Jensen and Chen, *J. Biol. Chem.*, **82**, 397 (1929); **87**, 741 (1930); Deulofeu, *Z. physiol. Chem.*, **237**, 171 (1935).

³¹⁶ Wieland, Konz, and Mittasch, *Ann.*, **513**, 1 (1934).

³¹⁷ Chen, Jensen, and Chen, *Proc. Soc. Exptl. Biol. Med.*, **29**, 905, 907 (1932); Hüttel and Behringer, *Z. physiol. chem.*, **245**, 175 (1937).

* Cholesterol and γ -sitosterol are the sterols that have been isolated from toad poisons. On the basis of the absorption spectra (p. 1271) of the sterol extracts it is possible that ergosterol is present, but the identification is not definite, since the other dehydrosterols show the same absorption bands. It is remarkable that a phytosterol should be present in the poison, and its occurrence is further proof that cholesterol is not the only animal sterol. The body sterol of the toad is cholesterol.

According to Kutscher and Ackermann [*Z. Biol.*, **84**, 181 (1926)], animals may be divided into two groups: the vertebrates who excrete creatinine, and the invertebrates who excrete arginine. (See also Baldwin, "An Introduction to Comparative Biochemistry," Macmillan, New York (1937), Chapter IV.) The toads form a third, special subgroup, since both free and combined arginine occurs in their poison glands—an indication of similarity of the toad to invertebrates and plants as opposed to the vertebrates. The poison of the toad may be viewed as another link to the plants and Hüttel and Behringer³¹⁷ have suggested that sitosterol may play a role in the genesis of these substances.

³¹⁸ Chen and Chen, *J. Pharmacol.*, **49**, 561 (1933).

³¹⁹ Abel and Macht, *ibid.*, **3**, 319 (1911); Jensen and Evans, Jr., *J. Biol. Chem.*, **104**, 307 (1934); Jensen, *J. Am. Chem. Soc.*, **59**, 767 (1937).

batting. The difficulties involved in obtaining the pure venoms and their genins are illustrated by the recent work of Wieland,³²⁰ in which the exudate from 33,000 toads was taken up in cotton batting, dried in vacuum, and the dried product extracted with chloroform. After purification of the chloroform extract by shaking out the acidic substances, the genins and related compounds were separated from fatty material by precipitation with petroleum ether. An acetone solution of the precipitate was then subjected to chromatographic analysis. In this way 36 g. of bufotalin and 29 g. of related substances were obtained, amounting to about 2 mg. of genins per toad. From the portion of the dried exudate not soluble in chloroform, 10.4 g. of bufotoxin, the conjugated poison, could be isolated.

From Ch'an Su or Senso, the dried secretion of the Chinese toad (*Bufo gargarizans*), two bufagins have been isolated. Kondo and Ikawa³²¹ obtained pseudobufotalin, $C_{24}H_{34}O_5(CH_3CO)$, m.p. 146°, and Tschesche³²² isolated cinobufagin, $C_{24}H_{32}O_5(CH_3CO)$, m.p. 223°. It is not clear why these two products should be obtained from the same dried secretion. Possibly different species of toads are involved.

The toad poisons are known to be members of the cyclopentanoperhydrophenanthrene group through the isolation of Diels' hydrocarbon from the products of selenium dehydrogenation of pseudobufotalin,³²³ of cinobufagin,³²² and of marinobufagin.³¹⁹ On the other hand, only impure chrysene has been obtained by selenium dehydrogenation of bufotalin.³²⁴ The formation of this hydrocarbon does not invalidate the argument, since it is also produced from other members of the group. These degradations are supported by x-ray measurements on bufagin, which show that the molecule is comparable to that of the sterols.³²⁵

A few of the structural details of the bufagins have been suggested by Wieland³²⁰ and by Tschesche.³²⁶ The genins and a number of the transformation products absorb light at 290–300m μ , and react with methanolic potassium hydroxide to form esters in the same way as scillaridin A. The two investigators have independently concluded that the lactone side chain is the same in the bufagins as in scillaridin A. An especially convincing argument is the similarity of the absorption curves of the bufagins and of the simple molecule, cumalinic acid³²⁶

³²⁰ Wieland, Hesse, and Hüttel, *Ann.*, **524**, 203 (1936).

³²¹ Kondo and Ikawa, *J. Pharm. Soc. Japan*, **53**, 2 (1933); [*Chem. Zentr.*, (II), 2558 (1933)]. The original work is in Japanese.

³²² Tschesche and Offe, *Ber.*, **68**, 1998 (1935); Jensen, *J. Am. Chem. Soc.*, **57**, 2733 (1935).

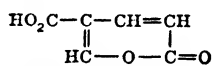
³²³ Ikawa, *J. Pharm. Soc. Japan*, **55**, 748 (1935); [*C. A.*, **29**, 7341 (1935)].

³²⁴ Wieland and Hesse, *Ann.*, **517**, 22 (1935).

³²⁵ Crowfoot, *J. Soc. Chem. Ind.*, **54**, 568 (1935).

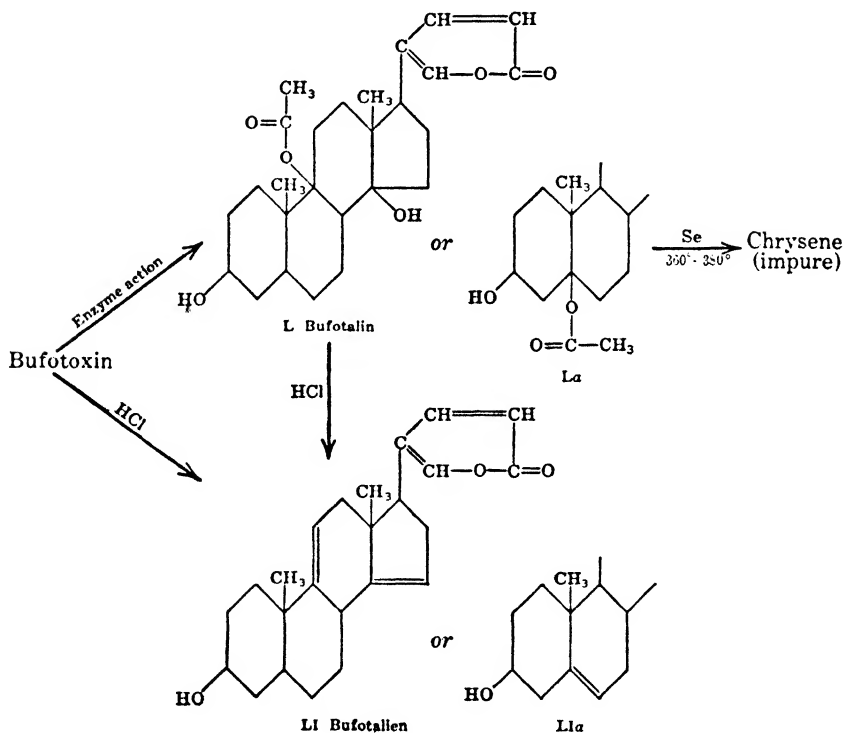
³²⁶ Tschesche and Offe, *Ber.*, **69**, 2367 (1936).

(XLIX). In accord with this formulation of the lactone side chain, ozonization of the bufagins gives glyoxylic and formic acids.³¹¹



XLIX Cumallic acid

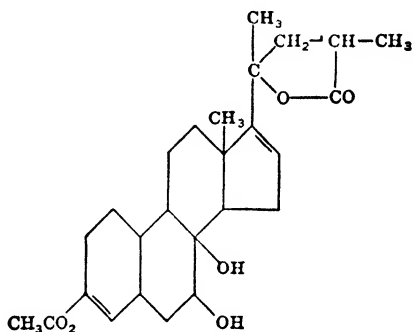
Bufotalin. Wieland³²⁰ has suggested structure L (or La) for bufotalin. This bufagin contains a secondary hydroxyl, a tertiary hydroxyl, and a tertiary acetoxy group. The tertiary hydroxyl and acetoxy groups are easily split off by cold hydrochloric acid to give the quadruply unsaturated bufotalien (LI or LIa). Since bufotalone, the



ketone from bufotalin, is isomerized when treated at 0° with dilute alkali, the tertiary hydroxyl group removed in the acid treatment is probably attached at C₁₄. The tertiary acetoxy group must be attached at C₉ or C₅, because the absorption spectrum of bufotalien (max. 290-300 mμ) does not indicate a conjugated system in the ring, and attachment at C₈ would lead to such a system. The secondary hydroxyl group is placed at C₃, as in the sterols.

Bufotalien is obtained when bufotoxin is hydrolyzed with hydrochloric acid. Wieland has suggested that the suberylarginine group is attached at the C_{14} —OH in the toxin, but the mode of linkage to the nitrogenous moiety is uncertain. On catalytic reduction of acetylbufotalien two products result—acetylbufotalan and a by-product, an acetylcholanic acid formed by reduction and fission of the lactone side chain. When this acetylcholanic acid is converted to a cholanic acid, isobufocholanic acid, m.p. 179° , $[\alpha]_D + 50.5$, is obtained.³²⁷ It is not identical with any of the known cholanic acids (Table IV).

Pseudobufotalin. A structure differing somewhat from the other bufagins has been developed by Ikawa^{323, 328} for pseudobufotalin (LII). By studying the products of Barbier-Wieland degradation, this worker has concluded that the side chain contains six carbon atoms, and



LII Pseudobufotalin
(provisional)

that a methyl group is missing at C_{10} . It is difficult to reconcile this work with that on the other bufagins.

Other Toad Poisons. In all, some twelve bufagins have been isolated. The most important are gamabufagin, $C_{24}H_{34}O_5$, m.p. 213° , from the dried skins of the Japanese toad;³²⁹ arenobufagin,³³⁰ $C_{23}H_{31}O_5$ — (CH_3CO) , m.p. 220° , from the Argentine toad; and regularobufagin,³³⁰ $C_{23}H_{31}O_5(CH_3CO)$, m.p. 236° , from the South African toad. The physical constants of these bufagins and the poisons have been tabulated by Dane³³¹ and Tschesche.²⁶⁹

³²⁷ Wieland, Hesse, and Meyer, *Ann.*, **493**, 272 (1932). For earlier work see Wieland and Weil, *Ber.*, **46**, 3315 (1913); Wieland and Alles, *Ber.*, **55**, 1789 (1922).

³²⁸ Ikawa, *J. Pharm. Soc. Japan*, **55**, 49 (1935); [*Chem. Zentr.*, (II), 1040 (1935)].

³²⁹ Wieland and Vocke, *Ann.*, **481**, 215 (1930).

³³⁰ Jensen, *J. Am. Chem. Soc.*, **57**, 1765 (1935). The earlier literature is cited in this publication.

³³¹ Dane, *Tabulae Biologicae Periodicae*, III, 204 (1933).

Structure and Physiological Action*

The effect on the diseased heart and the emetic action are the important physiological properties of the cardiac principles. Their structure and activity can be correlated in a general way. Diuresis is caused by the administration of these substances, but this is due more to an increased rate of blood circulation than to direct action on the kidney. Both the glycosides and the toad poisons are bitter in taste and irritating to the mucous membrane. Because of the latter property most of them produce diarrhea. Uzarin does not fit into the general pattern, however, since it is used to combat diarrhea.³³²

The Heart Action. The potency and usefulness of the cardiac glycosides are dependent on the presence of the unsaturated lactone ring, and are modified by the spatial configuration of rings A/B in the genin molecule and by the sugar moiety. The cardiotonic properties of these drugs are due to a direct action on the heart muscle. The response obtained with healthy muscle differs somewhat from that with diseased, so that animal experimentation is not directly comparable with clinical experience. Experimentally the physiological potency is determined by injecting an aqueous-alcoholic solution of the glycoside into the blood stream of cats (Hatcher-Brody method), or an aqueous solution in the lymph sac of frogs (U.S.P. method). The smallest amount of substance necessary to produce systolic standstill of the heart is determined, and the so-called minimum lethal dose (M.L.D.) calculated per kilogram of cat or per gram of frog. The frog method is generally employed, but the cat method is more satisfactory. In Table VIII the data of Chen³³³ on several of the pure glycosides are given. No values are reported for the weakly active hydrogenated glycosides in which the lactone ring is saturated. That a *cis* configuration of rings A B is essential for high potency is brought out in the values for digitoxin, thevetin, and uzarin. In uzarin the structure of the genin is comparable to that of dihydrocholesterol, and the potency of the glycoside both in the cat and the frog test is far lower than that of either of the others. Digitoxin has a configuration like that of *epicoprosterol*, and thevetin like *coprosterol*. Since digitoxin is more potent in the cat test and less potent in the frog test than thevetin, it is difficult to evaluate the effect of epimerization of the C₃—OH on the physiological activity.

In the cat test the activities of sarmentocymarin and digoxin are

* Books on pharmacology: Cushny, "Digitalis and Its Allies," Longmans, Green & Co., London (1925); Weese, "Digitalis," Thieme, Leipzig (1936).

³³² Windaus and Haack, *Ber.*, **63**, 1377 (1930).

³³³ Chen, Chen, and Anderson, *J. Am. Pharm. Assoc.*, **25**, 579 (1936).

TABLE VIII

THE PHYSIOLOGICAL POTENCY OF THE CARDIAC GLYCOSIDES

(Expressed in milligrams per kilogram of cat or milligrams per gram of frog)

Drug	Cat Units	Frog Minimal Systolic Dose	Minimal Emetic Dose in Cats
Convallatoxin.....	0.08	0.00021	0.060
β -Antiarin.....	0.10	0.00039	0.040
Ouabain.....	0.12	0.00050	0.060
Cymarin.....	0.13	0.00060	0.080
Scillaren A.....	0.15	0.00070	0.100
Sarmentocymarin.....	0.21	0.00545	0.09
Digoxin.....	0.22	0.00250	0.075
Digitoxin.....	0.33	0.00800	0.150
Thevetin.....	0.92	0.00450	0.225
Uzarin.....	5.08	1.50000	0.350

nearly the same, but in the frog test digoxin is about twice as potent as sarmentocymarin. The genins of these two glycosides differ only in the configuration of the hydrogen at C₉ and it follows that on a weight basis inversion at this point does not markedly affect the potency. The difference in activity is more clearly brought out when the results are considered on the basis of moles of glycoside per unit weight of animal. The molecular weight of sarmentocymarin is considerably less than that of digoxin and, mole for mole, sarmentocymarin is definitely less potent than digoxin. Thus inversion at C₉ decreases the heart activity.

The sugar moiety affects the absorbability of the glycosides from the intestine and determines the duration of the action. Those glycosides that are easily desugared *in vitro* are generally not satisfactory for medicinal use. For example, the strophanthin glycosides are not suitable for oral administration, but can be given intravenously when a rapid response is desired. Because a rapid response is seldom necessary, this kind of glycoside is rarely used; more often the glycosides of the digitalis group are employed—generally in the form of the dried leaf.

The Emetic Action. The minimum amount of glycoside or poison per kilogram of cat necessary to produce vomiting when given intravenously is called the emetic dose. It is uncertain how the drugs produce this response, but the action does not take place on the vomiting center of the brain.* As the data of Table VIII show, the correlation

* For a critical discussion see Weese, "Digitalis," Thieme, Leipzig (1930).

between cardiatic potency and the emetic dose is poor, although qualitatively the order of the drugs in the two effects is nearly the same.

THE DIGITALIS SAPOGENINS ³³⁴

The saponins are a group of glycosides with the ability to produce stable foams when their aqueous solutions are shaken. The cardiac glycosides also produce foams and are saponins, but, because of their characteristic heart action, they are treated as a separate class. The saponins occurring with the cardiac glycosides of the digitalis group are differentiated from the others by the designation "the digitalis saponins." This separation is chemically correct, for the digitalis saponins contain the cyclopentanoperhydrophenanthrene nucleus and yield Diels' hydrocarbon when dehydrogenated with selenium, whereas most of the other saponins are built up on some unknown ring system and yield 1,2,7-trimethylnaphthalene (sapotalene) when dehydrogenated.³³⁵

Like the cardiac glycosides the digitalis saponins taste bitter and are irritating to the mucous membranes. Given intravenously they are poisonous, but taken orally they are non-toxic, probably because they are not absorbed in the intestine. The poisonous properties of these glycosides are more pronounced toward lower forms of animal life than higher. For this reason crude extracts of the saponins have been used by primitive peoples to catch fish. The extracts are poured into streams and the fish are either stunned or killed by the glycoside. Since the saponins are not harmful when taken internally, fish killed in this way are edible. One of the most interesting characteristics of the saponins is their ability to hemolyze red blood corpuscles in very low concentration. The dilution (or index) for these various physiological effects is of the same order of magnitude; the dilutions for the saponin, digitonin, are given below:

Taste index ³³⁶	1 : 380,000	Fish index ^{336,338}	1 : 200,000
Eye index ^{336,337}	1 : 230,000	Hemolytic index ³³⁹	1 : 168,000

The hemolytic action of the digitonin, for example, has been attributed to its ability to form a molecular compound with the chole-

³³⁴ Books: Fieser; Lettré and Inhoffen; Kofler, "Die Saponine," Springer, Vienna (1927). Reviews: Tachesche, *Angew. Chem.*, **48**, 569 (1935); *Ergeb. Physiol.*, **38**, 65 (1936).

³³⁵ Cf. Ruzicka *et al.*, *Helv. Chim. Acta*, **15**, 431, 1496 (1932); **17**, 442 (1934).

³³⁶ Kofler and Schrutka, *Biochem. Z.*, **159**, 327 (1925).

³³⁷ Kobert, *Arch. expil. Path. Pharmacol.*, **23**, 257 (1887).

³³⁸ Kofler, *Biochem. Z.*, **129**, 64 (1922). The fish index is usually defined as that dilution required to kill fish weighing 0.1-0.5 g. A species of minnow (Rotaue) is used for the assay.

³³⁹ See Kofler, reference 334. The value given is for human blood.

terol of the red cell, and to bring about permeability in this way. This mechanism appears to be incorrect, since there is no parallel between the cholesterol content of the cells and their resistance to hemolysis.³⁴⁰ Cholesterol digitonide, on the other hand, is devoid of hemolytic properties.

The digitalis saponins form solid molecular compounds with the higher alcohols, phenols, and thiophenols.³⁴¹ The addition compounds formed with digitonin have been studied more thoroughly than those formed with the other saponins, and in all cases the ratio of saponin to alcohol, or phenol, is 1 : 1. The addition compounds are insoluble in water and generally soluble in alcohol. Their solubility in alcohol, however, varies with the nature of the molecule and the steric configuration of the hydroxyl group.* The determination of the configuration of the C₃—OH group in the steroids and the separation of optical antipodes in this and other³⁴² groups are important applications of the reagent. The use of digitonin has been limited by its cost.

The Digitalis Saponins. The recognized members of the digitalis saponins are shown in Table IX. Preparation of these glycosides in pure form is a tedious and uncertain process. From the crude extracts of the leaves and seeds of the digitalis family the cardiac glycosides can be removed by means of chloroform or ether. The separation of digitonin from the mixture of digitonin, gitonin, tigonin, and other saponins obtained from *D. purpurea* illustrates the procedures employed. By the method of Kiliani³⁴³ the digitonin is precipitated from an aqueous solution as the amyl alcohol addition product, regenerated by removal of the amyl alcohol, and the product recrystallized first from 50 per cent and then from 85 per cent alcohol. Windaus and Shah³⁴⁴ precipitate the digitonin from a 5 per cent aqueous solution by the addition of ether with which it forms an addition compound. The precipitate is filtered off at the end of thirty minutes; repetition of the

³⁴⁰ See Ponder, "The Mammalian Red Cell and the Properties of Hemolytic Systems," Borntraeger, Berlin (1934), Chapter VIII.

³⁴¹ Windaus, *Ber.*, **42**, 238 (1909); Windaus and Weinhold, *Z. physiol. Chem.*, **126**, 299 (1923).

* Digitonin is adsorbed as a visible film on monomolecular layers of sterols, but only slightly by *epi*-sterols. The normal sterols give very rigid films which expand slowly, but the *epi*-sterols form rapidly expanding liquid films. The digitonide films from the *epi*-sterols are more unstable than those of the normal sterols and are hydrophobic; the normal sterol digitonides are hydrophilic [Langmuir and co-workers, *J. Am. Chem. Soc.*, **59**, 1406, 1751 (1937)].

³⁴² Windaus, Klänhardt, and Weinhold, *Z. physiol. Chem.*, **126**, 308 (1923).

³⁴³ Kiliani, *Ber.*, **43**, 3562 (1910); **49**, 701 (1916). A summary of the method is given by Lettré and Inhoffen.

³⁴⁴ Windaus and Shah, *Z. physiol. Chem.*, **151**, 86 (1926); cf. Windaus and Schneckenburger, *Ber.*, **46**, 2628 (1913).

process gives pure digitonin. By the last procedure gitonin is also obtained, since it precipitates with ether on standing.

It is very difficult to separate tigonin from the mixtures of gitonin and tigonin that occur in nature. Fortunately the leaves of *D. lanata* contain only tigonin, and the saponin is easily purified through its sparingly soluble cholesterol addition product.³⁴⁵

Two other saponins have been isolated—sarsasaponin from the Mexican sarsaparilla root, *Radix sarsaparillae*,³⁴⁶ and amolonin from the California soap plant or amole, *Chlorogalum pomeridianum*.³⁴⁷ The occurrence of these saponins in plants other than the digitalis group indicates that the designation "the digitalis saponins" is not ideal.

The Sapogenins. By acid hydrolysis the saponins are split to sugars and sapogenins. The hydrolytic products are given in Table IX,

TABLE IX
DIGITALIS SAPONINS

Saponin	Probable Formula	Plant Source	Hydrolytic Products	
			Sapogenin	Sugars
Sarsasaponin (parillin)	$C_{44}H_{74}O_{17}$	<i>Radix sarsaparillae</i>	Sarsasapogenin (parigenin)	2 Glucose and 1 rhamnose
Gitonin	$C_{41}H_{72}O_{15}$	<i>Digitalis purpurea</i>	Gitogenin	3 Galactose and 1 pentose
Digitonin	$C_{46}H_{82}O_{19}$	<i>Digitalis purpurea</i>	Digitogenin	4 Galactose and 1 xylose
Tigonin	$C_{46}H_{82}O_{21}$	<i>Digitalis purpurea</i> , <i>Digitalis lanata</i>	Tigogenin	2 Glucose, 2 galactose, and rhamnose
Amolonin	$C_{63}H_{104}O_{31}$	<i>Chlorogalum pomeridianum</i>	Tigogenin	3 Glucose, 1 galactose, and 2 rhamnose

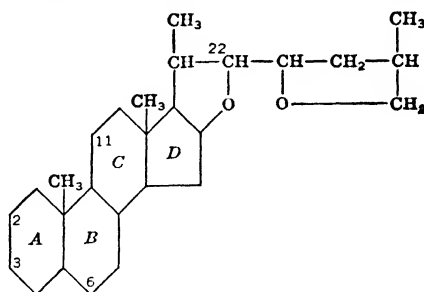
and, as this list shows, the sugars present in the glycosides are not unique. The general structure of the sapogenins given in formula I indicates that they are comparable to the sterols with the difference that the principal side chain at C_{17} contains two hydrofuran rings, one

³⁴⁵ Tschesche, *Ber.*, **69**, 1665 (1936).

³⁴⁶ Jacobs and Simpson, *J. Biol. Chem.*, **105**, 501 (1934).

³⁴⁷ Jurs and Noller, *J. Am. Chem. Soc.*, **58**, 1251 (1936); cf. Liang and Noller, *ibid.*, **57**, 525 (1935).

of which is fused with ring D. The structure of the side chain has not been established with absolute certainty and must be regarded as provisional. The several sapogenins differ in the number and position of the secondary hydroxyl groups that are attached to this ring system, in the relationship of rings A/B to each other, and possibly in the steric



I Ring System of the digitalis sapogenins
(provisional)

Rings A/B; *trans* or *cis*. The nucleus is hydroxylated at one or more of the numbered positions.

configuration of the C₁₇ side chain. The positions of substituent hydroxyl groups and the physical properties of the sapogenins are shown in Table X.

TABLE X
DIGITALIS SAPOGENINS

Sapogenin	Position of Hydroxyl Groups (cf. Formula I)	Formula	M. P., ° C.	[α] _D	Source
Sarsasapogenin (parigenin)	3 (?)	C ₂₇ H ₄₄ O ₃	199	—60 (MeOH)	Sarsasaponin
Smilagenin.....	3 (?)	C ₂₇ H ₄₄ O ₃	183-184	—61 (MeOH)	Jamaica sarsa- parilla root
Tigogenin.....	3	C ₂₇ H ₄₄ O ₃	204	—49 (pyridine)	Tigonin
Chlorogenin....	C ₂₇ H ₄₄ O ₄ (?)	276	<i>Chlorogalum</i> <i>pomeridianum</i>
Gitogenin.....	2,3	C ₂₇ H ₄₄ O ₄	272	Gitonin
Digitogenin....	2,3,6	C ₂₇ H ₄₄ O ₅	280-283	—81 (CHCl ₃)	Digitonin

Although the sapogenins are easily purified through the acetyl compounds, their composition as compounds containing 27 carbon

atoms was not established until 1935, since combustion analysis by the older methods did not differentiate between C_{26} and C_{27} . It remained for Jacobs and Simpson³⁴⁸ to alter the accepted compositions and at the same time to show the relationship of the sapogenins to the sterols by dehydrogenating gitogenin and sarsasapogenin with selenium to Diels' hydrocarbon. Another product of selenium dehydrogenation is a hexyl methyl ketone that appears to be isohexyl methyl ketone.³⁴⁹ The same or a similar ketone is obtained when the sapogenins are treated with an acetic acid solution of hydrochloric acid. When these facts were combined the structural pattern of the sapogenins began to emerge. A later stage in the structural development was the degradation of tigogenin to etioallocholanolic acid and sarsasapogenin to etiocholanolic acid. The nature of the side chain has been imperfectly determined, however, and must be studied further. Surface film measurements confirm the general structure given in formula I, but naturally are not helpful in establishing the structure of the side chain.³⁵⁰

Tigogenin. Of the three oxygen atoms contained in the molecule of tigogenin, $C_{27}H_{44}O_3$, one is present as a secondary hydroxyl group and the other two in ether linkages. These groupings have been placed, in part, by the application of the methods used for other members of the cyclopentanoperhydrophenanthrene group.³⁵¹ The ring system was characterized by chromic acid oxidation of acetyltigogenin (II) to an acetylated lactone (III), reduction of the secondary hydroxyl group to the lactone IV, and Barbier-Wieland degradation through the compound of structure V to a mixture of etioallobilanic acid (VI) and the lactone of probable structure VII. As a result of this degradation the relationship of rings A/B is shown to be *trans*, the attachment of the principal side chain is established at C_{17} , and one of the ether oxygens is placed at C_{16} , extending to form a bridge to C_{22} , or possibly to C_{23} . Attachment at C_{22} though not definitely proved seems to be highly probable. The lactone ring of structure IV is definitely a five-membered ring, since it is opened with difficulty by alkali in the cold, and the hydroxy acid formed immediately reverts to the lactone form in the presence of acid.

The structure of the portion of the side chain that is burnt off is more difficult to reconstruct. The evidence for this is discussed in connection with the structure of digitogenin.

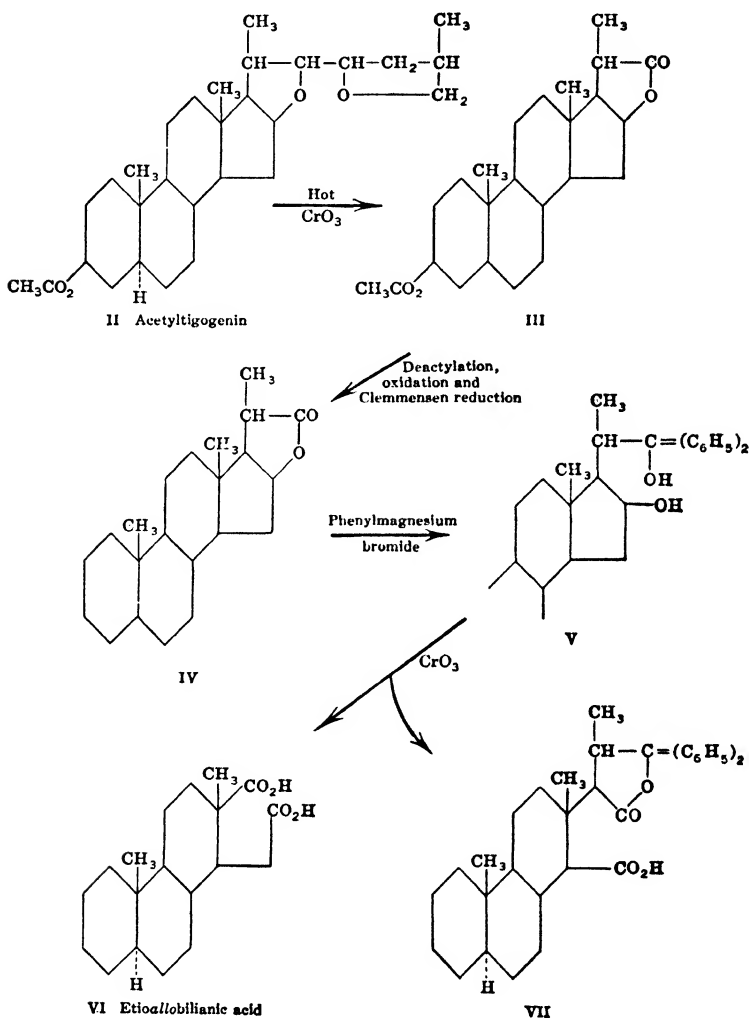
³⁴⁸ Jacobs and Simpson, *ibid.*, **56**, 1424 (1934); *J. Biol. Chem.*, **105**, 501 (1934).

³⁴⁹ Ruzicka and van Veen, *Z. physiol. Chem.*, **184**, 69 (1929); Simpson and Jacobs, *J. Biol. Chem.*, **109**, 573 (1935).

³⁵⁰ Askew, Farmer, and Kon, *J. Chem. Soc.*, 1399 (1936).

³⁵¹ Tschesche and Hagedorn, *Ber.*, **68**, 1412 (1935).

The attachment of an hydroxyl group in ring A in tigogenin has been established in the usual way by opening the ring to which the hydroxyl is attached, and subjecting the product to thermal decomposition. Since a pyro ketone is formed, and tigogenin forms an insoluble



digtonide, the hydroxyl group must be attached at C₃, probably *cis* to the C₁₀—CH₃. To rule out the possibility of attachment at C₄, Tschesche³⁵² prepared the two isomeric 4-hydroxycholestanes and found that neither gives an insoluble digtonide.

³⁵² Tschesche and Hagedorn, *Ber.*, **68**, 2247 (1935).]

Gitogenin. The sapogenin, gitogenin, contains two secondary hydroxyl groups in an α -glycol relation. These hydroxyl groups have been placed at C₂ and C₃ through correlation with tigogenin.³⁵³ When gitogenin is oxidized with chromic acid in the cold, the dicarboxylic acid, gitogenic acid is formed. This acid is identical with the product formed from tigogenin by chromic acid oxidation at 70°. Since tigogenin has an *allo* structure and the dicarboxylic acid is formed by the opening of ring A, the diacid must result from the genin by cleavage between C₂ and C₃ (p. 1254). The formation of this acid from gitogenin can be reconciled only with the attachment of the two secondary hydroxyl groups at C₂ and C₃.

Gitogenic acid has one unusual reaction. The dimethyl ester of gitogenic acid is not difficult to saponify, but one ester group is hydrolyzed more easily than the other.³⁵⁴ It is difficult to reconcile this fact with the formulation of hydroxyl groups at C₂ and C₃, since the carbomethoxy groups of the resulting acid should be equally easily hydrolyzed.

Digitogenin. When digitogenin (VIII), which contains three secondary hydroxyl groups, is oxidized with cold chromic acid one of the products is digitogenic acid (IX), a ketodicarboxylic acid. On reduction of the carbonyl group of this acid by the Wolff-Kishner method, gitogenic acid is formed.³⁵³ Thus two of the hydroxyl groups of digitogenin are placed at C₂ and C₃, as in gitogenin.³⁵⁵

If digitogenic acid is oxidized with permanganate, ring B is opened and a ketotricarboxylic acid (X), "oxydigitogensäure," is formed.³⁵⁶ This acid is a β -ketonic acid since it readily loses one or two molecules of carbon dioxide when it is heated. The probable structure of the acid formed by the loss of one molecule of carbon dioxide is shown in structure XI. The second molecule of carbon dioxide is split out as indicated by the dotted lines. The formation of acid X shows that the unplaced hydroxyl group is situated near the bridge head of two condensed rings. The position of this hydroxyl group, or the carbonyl produced from it, is further defined by the rearrangement of digitogenic acid to digitonic acid (XII) by warming with alkali. Taking both of these reactions into consideration, the third secondary hydroxyl group is placed at C₆.³⁵⁵

The structure of the side chain is more difficult to establish. From the work of Kiliani^{355,357,358} it is known that methylsuccinic acid is

³⁵³ Tschesche, *Ber.*, **68**, 1090 (1935)

³⁵⁴ Jacobs and Simpson, *J. Biol. Chem.*, **110**, 429 (1935).

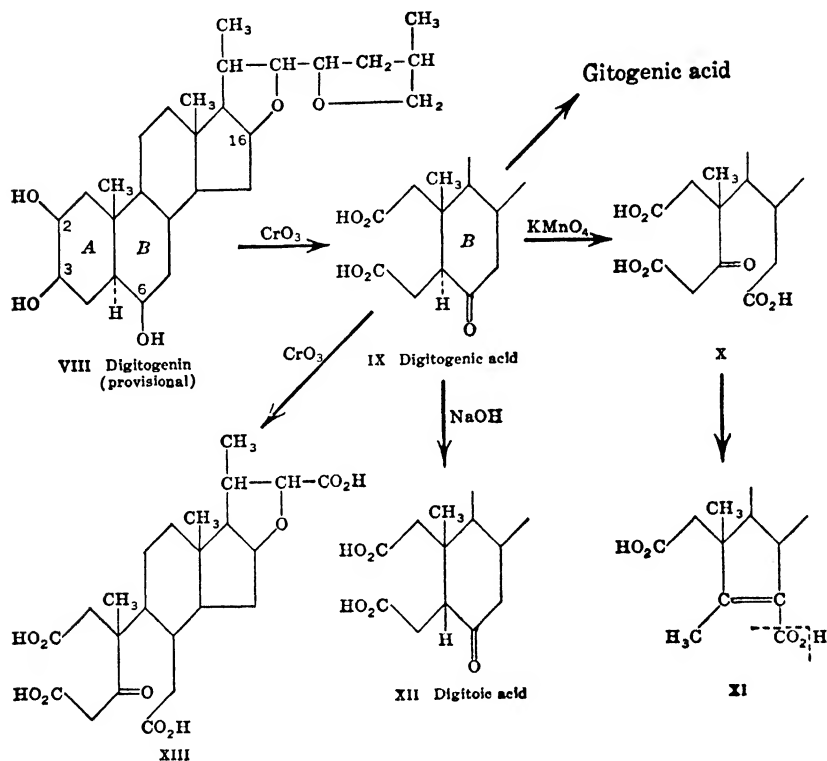
³⁵⁵ Tschesche and Hagedorn, *Ber.*, **69**, 797 (1936).

³⁵⁶ Windaus and Weil, *Z. physiol. Chem.*, **121**, 62 (1922).

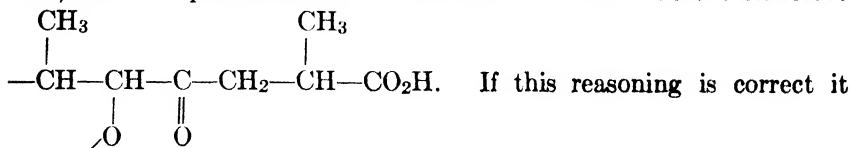
³⁵⁷ Windaus and Willerding, *ibid.*, **143**, 33 (1925).

³⁵⁸ Kiliani, *Ber.*, **49**, 702 (1916).

one of the products of chromic acid oxidation of gitogenin and digitogenin. The formation of this acid can best be reconciled with a degradation of the side chain and indicates a methyl branching in the chain. Further evidence for the structure of the side chain comes from the acids that are obtained from the sapogenins as by-products on oxidation



with cold chromic acid.^{355, 357, 358} Apparently the oxide ring of the terminal portion of the chain is opened, a methyl or methylene group is converted to carboxyl, and the newly formed carboxyl group lactonizes with the carbonyl group formed from the oxygen that was formerly a part of the oxide ring. This behavior is characteristic of a γ -ketonic acid, and it is probable that the oxidized side chain has the structure

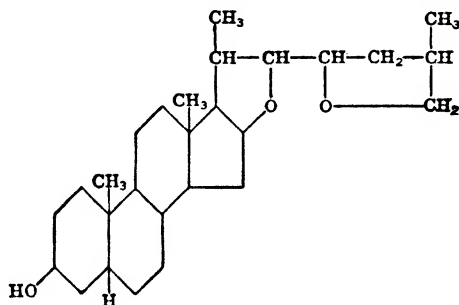


should be possible to isolate an oxidation product in which cleavage at the γ -carbonyl has taken place. This has been realized in the case of

compound XIII, which is formed by chromic acid oxidation of digitogenic acid. Like the structure of compound X, that of XIII has not been rigorously established, but it is compatible with the known reactions.

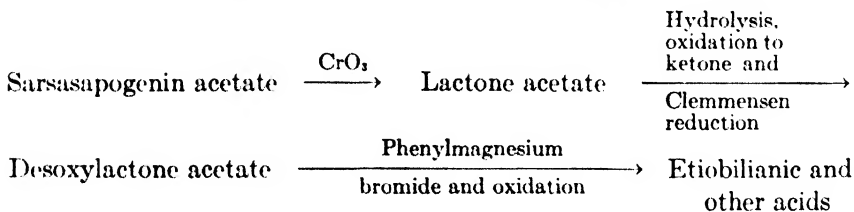
The side chain warrants further study. In particular, the reactions with nitric acid³⁴⁶ and the cleavage of the side chain to a hexyl methyl ketone by the action of a mixture of acetic and hydrochloric acid³⁵⁹ are difficult to interpret.

Sarsasapogenin.³⁶⁰ A provisional structure for sarsasapogenin is given in formula XIV. This formulation shows that the genin differs from tigogenin only in relationship of rings A/B, but the structure of the side chain and the position of the hydroxyl group have not been



XIV Sarsasapogenin
(provisional)

definitely determined. The ring system of sarsasapogenin has been established by oxidative degradation analogous to that employed with tigogenin. The degradation was carried out through the stages:



The formation of etiobilanic acid (p. 1244) shows that the relationship of rings A/B is *cis* in this genin. Since the genin forms an insoluble digitonide and surface film measurements indicate that its structure is comparable to that of the other sapogenins, the hydroxyl group of sarsasapogenin is probably attached at C₃. Simpson and Jacobs,³⁶⁰ however, have placed the hydroxyl group at C₁₁ as a result of the study

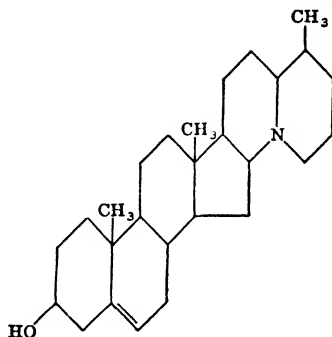
³⁵⁹ Simpson and Jacobs, *J. Biol. Chem.*, **109**, 573 (1935).

³⁶⁰ Simpson and Jacobs, *ibid.*, **109**, 573 (1935); **110**, 565 (1935); Askew, Farmer, and Kon, *J. Chem. Soc.*, 1399 (1936); Farmer and Kon, *ibid.*, 414 (1937).

of sarsasapogenic acid and sarsasapogenone. Sarsasapogenic acid is formed by opening the ring bearing the hydroxyl group and on thermal decomposition an anhydride is produced rather than a ketone. Sarsasapogenone, the ketonic intermediary product in the conversion to sarsasapogenic acid, is resistant to reduction. Neither of these facts is compatible with the attachment of the hydroxyl group at C₃.

Other Sapogenins. Two other sapogenins have been reported: chlorogenin³⁶¹ from the California soap plant (or amole), and smilagenin³⁵⁰ from the Jamaica sarsaparilla root. Little is known of chlorogenin, but Farmer and Kon³⁶⁰ have oxidized smilagenin to the same lactone acetate as that obtained by chromic acid oxidation of sarsasapogenin acetate. Evidently smilagenin differs from sarsasapogenin in the steric configuration of the C₁₇ side chain.

Solanidine. A nitrogen-containing glycoside, solanine, *C₄₆H₇₃O₁₅N, has been isolated from potato sprouts.³⁶² Since the genin of this glycoside contains the cyclopentanoperhydrophenanthrene nucleus, solanine is interesting as a bridge between the alkaloids and the members of this group. On hydrolysis the glycoside yields solanidine, C₂₇H₄₃ON, m.p. 216°, and one molecule each of glucose, galactose, and rhamnose.



XV Solanidine
(provisional)

When dehydrogenated with selenium, solanidine gives Diels' hydrocarbon. The genin contains one double bond, a secondary hydroxyl group, and apparently three C—CH₃ groups; it forms an insoluble digitonide. The structure XV that has been suggested³⁶³ for solanidine shows a

³⁶¹ Liang and Noller, *J. Am. Chem. Soc.*, **57**, 525 (1935).

* Other solanines are known but poorly characterized. The several glycosides are differentiated by the first letter of the botanical name of the plant order from which each is derived. Thus the solanine from potato (*Solanum tuberosum*) sprouts is more precisely named solanine *t*.

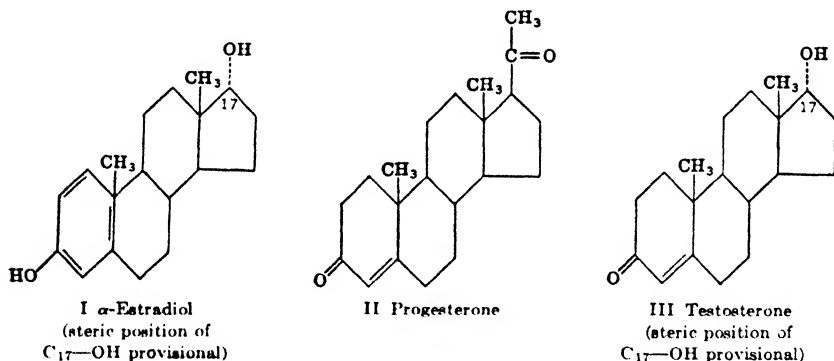
³⁶² Oddo and Caronna, *Ber.*, **67**, 446 (1934).

³⁶³ Soltys and Wallenfels, *Ber.*, **69**, 811 (1936).

tertiary nitrogen in a ring system condensed on ring D. This is based on the fact that N-alkyl derivatives cannot be formed.

THE SEX HORMONES ³⁶⁴

It has been known for years that the glands of the genital systems elaborate substances that cause important physiological changes. By studying the results of castration and castration followed by implantation of the glands removed or of those of the opposite sex, physiologists were able to determine some of the effects due to secretions of the genital glands of the two sexes. A further stage was reached when the effect of extracts of the gonads on castrated animals was studied. Through these methods definite bioassays have been developed for the evaluation of the several hormones. At the present time three types of sex hormones are recognized as originating in the gonads: the estrogenic hormones, the hormone of the corpus luteum, and the androgenic hormones. The structures of the glandular hormones are shown in formulas I-III. α -Estradiol (I) is the ovarian hormone that produces



estrus; progesterone (II), the corpus luteum hormone that is essential for pregnancy; and testosterone (III), the testicular hormone that causes changes in the accessory sexual organs of the male. The production of these sex hormones appears to be regulated by the gonadotropic hormones secreted in the anterior lobe of the pituitary. That these hormones from the pituitary are responsible for the production of the sex hormones has been shown by the removal of the gland and by the injection of extracts.

³⁶⁴ Books: Fieser; Lettré and Inhoffen. General reviews: Ruzicka, *Helv. Chim. Acta* **19**, E89 (1936); Butenandt, *Naturwissenschaften*, **24**, 529 (1936). Physiology: Allen, "Sex and Internal Secretions," Williams and Wilkins Co., Baltimore (1932); Zondek, "Die Hormone des Ovariums und des Hypophysen Vorderlappen," 2nd ed., Springer, Vienna, (1935); Houssay, "Ann. Rev. Biochem.," Vol. IV, p. 279 (1935).

The Estrogenic Hormones³⁶⁵

In the female sex organs a periodic change takes place which varies somewhat from species to species. As the result of the action of the estrogenic hormones the female is brought into a state of heat (estrus), during which she will mate. In estrus, rats, mice, and guinea pigs show characteristic changes in the tissues of the vagina accompanied by a typical vaginal discharge that has a unique cornified appearance. By microscopic examination of the vaginal smears from such an animal the estrous condition is easily recognized. Allen and Doisy,³⁶⁶ using castrated female rats and mice, adapted this phenomenon to a biological assay of estrogenic activity. At the present time the assay is carried out by injecting subcutaneously into a group of five or more castrated mice (or rats) several concentrations of the substance under examination. A group of control animals is simultaneously injected with a standard estrogenic substance. By comparison of the concentrations necessary to produce estrus in more than 50 per cent of the animals the assay can be made with some precision. The results are generally expressed in mouse units (M.U.); by international agreement one mouse unit is defined as the effect produced by 0.1 γ of a standard estrone preparation.³⁶⁷ As a subsidiary standard the monobenzoyl ester of α -estradiol (I) is used; this unit (benzoate unit) is represented by the specific activity contained in 0.1 γ of the ester.³⁶⁸

Occurrence. It was not until the development of the Allen-Doisy vaginal smear technique of studying estrus that sources of the hormone could be examined. Since then the estrogens have been found to be present in the gonads and the placenta, but these organs have a low hormonal content. The hormones are eliminated in the urine of both sexes and although the content of pregnancy urine is high, the urines of the stallion and other males of the Equidae are the richest sources known. In Table XI representative values of the several urines are given.³⁶⁹ Estrogenic hormones have been found in the lower forms of animal life and in the plant kingdom. Thus, one of the hormones—estrone—has been isolated from palm kernel extract,³⁷⁰ and another—

³⁶⁵ Reviews: Marrian, *Physiol. Rev.*, **13**, 185 (1933); Störmer and Westphal, *Ergeb. Physiol.*, **35**, 318 (1933); Wade, in "Fortschritte der physiol. Chem., 1929-1934," Junk, Berlin (1934), p. 277. Physical Data: Butenandt, *Tabulae Biologicae Periodicae*, **III**, 202 (1933).

³⁶⁶ Allen and Doisy, *J. Am. Med. Assoc.*, **81**, 819 (1923).

³⁶⁷ Lormand, *Bull. soc. chim. biol.*, **15**, 1566 (1933).

³⁶⁸ Gautier, *League of Nations Quart. Bull. of the Health Organization*, **IV**, 543 (1935).

³⁶⁹ Borchardt, Dingemans, and Laqueur, *Naturwissenschaften*, **22**, 190 (1934); Zondek, *Nature*, **133**, 209 (1934).

³⁷⁰ Butenandt and Jacobi, *Z. physiol. Chem.*, **218**, 104 (1933).

TABLE XI
CONTENT OF ESTROGENIC HORMONE OF VARIOUS URINES

	Normal		Pregnant	
	M. U. per Liter	M. U. per Diem	M. U. per Liter	M. U. per Diem
Woman	425	600	21,000	31,000
Man	160	240		
Mare	200	2,000	100,000	1,000,000
Stallion	170,000	1,700,000		
Zebra (male)	36,000			
Bull	330			

estriol—from pussywillows.³⁷¹ Potent extracts have been obtained from a wide variety of sources including petroleum and coal tar,* but it is uncertain whether the activity is due to true hormones or to other compounds.

Isolation. The procedure used in the isolation and purification of the estrogenic hormones is rather complex, since it requires a concentration of about a millionfold and a separation from substances that are physically similar. Pregnancy urine of women or of mares, or the urine of stallions, is the usual source. In the urine the hormones are present to some extent as glucuronates³⁷² from which they are liberated by boiling after the addition of concentrated hydrochloric acid. The hormones may then be extracted with organic solvents, the extracts freed of acidic impurities (auxin *a*, etc.), and purified to a high degree by partition between various solvents; or a new reagent, trimethylamino-acetohydrazide hydrochloride (Girard's reagent T),³⁷³ may be employed at this stage. This hydrazide reacts with ketones to form water-soluble derivatives, and since the estrogenic compounds in urine are ketonic in nature, they are easily separated from fats and other substances. The final purification is effected by distillation in high vacuum followed by recrystallization. Addition compounds, such as quinoline with estrone, or the acid half-esters formed with phthalic anhydride have been employed for the final purification. By another procedure the hormones

³⁷¹ Skarzynski, *Nature*, **131**, 766 (1933).

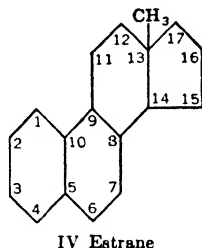
* For list see monograph of Lettré and Inhoffen, pp. 252-253. Among the unusual sources of potent estrogenic substances is the mud of the Dead Sea [Zondek, *Nature*, **140**, 240 (1937)].

³⁷² Cohen and Marrian, *Biochem. J.*, **30**, 57 (1936).

³⁷³ Girard and Sandulesco, *Helv. Chim. Acta*, **19**, 1095 (1936).

are adsorbed from the urine and the adsorbate worked up after elution in much the same manner as that portrayed above.*

General Properties. Some seven estrogenic hormones have been isolated from urine and the tissues of the genital system, and apparently there are hormones in addition to those that are now known.³⁷⁴ The physical properties and physiological potencies of these hormones are listed in Table XII. The estrogens may be named as derivatives of the



hypothetical hydrocarbon estrane³⁷⁵ (IV). All the hormones are crystalline phenolic compounds and often occur in polymorphic modifications;³⁷⁶ they absorb strongly in the ultra-violet at 280–285 mμ.³⁷⁷ Surface film and x-ray measurements early suggested that the hormones were structurally similar to the sterols.³⁷⁸ Later work confirmed this hypothesis, but brought out the difference that in the hormones ring A is benzenoid. The phenolic hydroxyl group has been placed at C₃ and a carbonyl group at C₁₇ in the estrogens obtained from urine. This carbonyl group is thought to be formed by the *in vivo* oxidation of the tissue hormones, e.g., α-estradiol (I). Probably the phenolic hydroxyl group is partly responsible for the color reaction that the estrogens give

* Literature on isolation: Butenandt and Hildebrandt, *Z. physiol. Chem.*, **199**, 243 (1931); Marrian, *Biochem. J.*, **23**, 1090, 1233 (1929); **24**, 435, 1021 (1930); Curtis, *J. Biol. Chem.*, **100** (Proc.) xxxiii (1933); Curtis, MacCorquodale, Thayer, and Doisy, *ibid.*, **107**, 191 (1934); Cartland, Meyer, Miller and Rutz, *ibid.*, **109**, 213 (1935).

³⁷⁴ Wintersteiner, Schwenk, Hirschmann, and Whitman, *J. Am. Chem. Soc.*, **58**, 2652 (1936).

³⁷⁵ Adam, *et al.*, *Nature*, **132**, 205 (1933). At the suggestion of Prof. A. M. Patterson this nomenclature has been modified by including parenthetically the linkage of the double bond from C₆.

³⁷⁶ Kofler and Hauschild, *Z. physiol. Chem.*, **224**, 150 (1934).

³⁷⁷ See Morton, "Absorption Spectra of Vitamins and Hormones," Hilger, London (1935), p. 64; cf. Rowlands and Callow, *Biochem. J.*, **29**, 837 (1935).

³⁷⁸ Bernal, *J. Soc. Chem. Ind.*, **51**, 259 (1932); Adam, Danielli, Haslewood, and Marrian, *Biochem. J.*, **26**, 1233 (1932); Danielli, Marrian, and Haslewood, *ibid.*, **27**, 311 (1933); Danielli, *J. Am. Chem. Soc.*, **56**, 746 (1934). The original measurements did not differentiate well between several possible structures and were at first misinterpreted.

TABLE XII
ESTROGENIC HORMONES

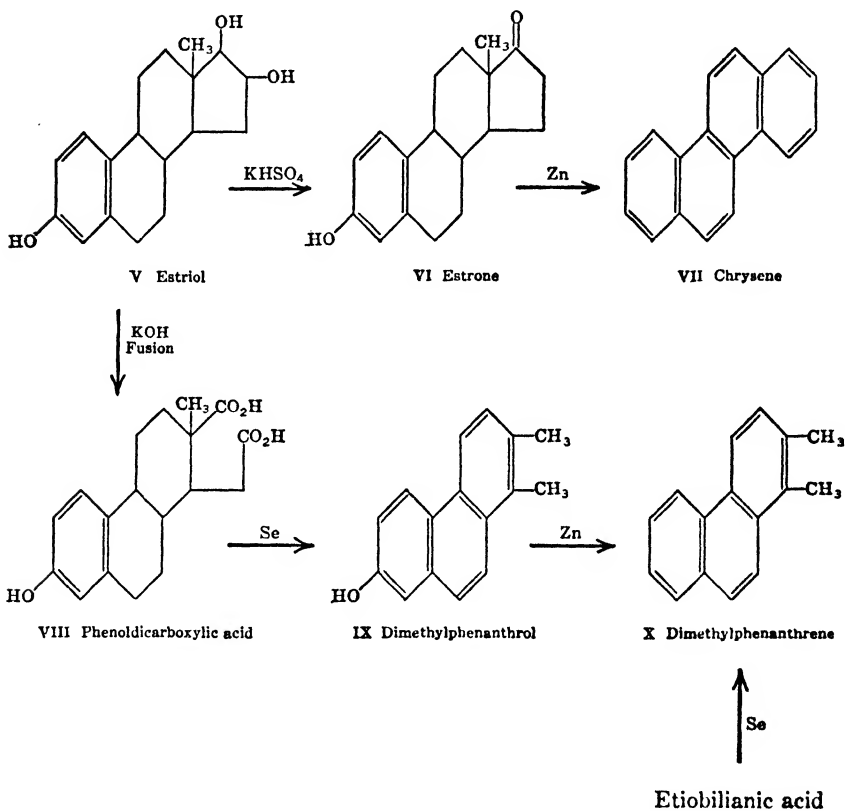
Hormone*	Structure†	Formula	M. P., ° C.	[α] _D (Dioxane)	Potency, Million Mouse Units per Gram	Source
Equilenin	3-Hydroxy-16-keto-1,3,5(10),6,8(9)-estrapentaene	$C_{18}H_{18}O_2$	258-259 c.	+87	0.4-0.7	Mares' urine
Dihydroequilenin	3,17-Dihydroxy-1,3,5(10),6,8(9)-estrapentaene	$C_{18}H_{20}O_2$	215-217 c.	-47	0.2-0.35 (?)	Mares' urine
Equilin	3-Hydroxy-17-keto-1,3,5(10),7-estratetraene (?)	$C_{18}H_{18}O_2$	238-240 c.	+308	1.2	Mares' urine
Hippulin	$C_{18}H_{20}O_2$	223 c.	+128	1.2	Mares' urine
Estrone	3-Hydroxy-17-keto-1,3,5(10)-estratriene	$C_{18}H_{22}O_2$	255 c.	+156	8	Pregnancy and stillion urine, amniotic fluid, palm nuts
α -Estradiol	3,17-Dihydroxy-1,3,5(10)-estratriene	$C_{18}H_{18}O_2$	178 c.	+80	13-15	Ovaries, reduction of estrone
β -Estradiol	3,17-Dihydroxy-1,3,5(10)-estratriene	$C_{18}H_{18}O_2$	223	+54	0.6-0.8	Reduction of estrone
Estriol	3,16,17-Trihydroxy-1,3,5(10)-estratriene	$C_{18}H_{24}O_3$	280	+30	0.075-0.5	Pregnancy urine, placenta

* Synonyms: Estrone: Follicular hormone, estrin, theelin. Estradiol: Dihydrofollicular hormone. Estriol: Follicular hormone hydrate, emmenin, theelol.

† See note of reference 375.

with sulfonic acids and other reagents; no coloration is produced with ferric chloride.*

Estrone and Estriol. The first of the estrogenic hormones to be isolated was estrone (VI). This was reported nearly simultaneously by Doisy³⁷⁹ and Butenandt.³⁸⁰ Later Marrian³⁸¹ discovered the trihydroxy compound estriol (V). The structural investigation of these compounds is due to the efforts of Butenandt,³⁸²



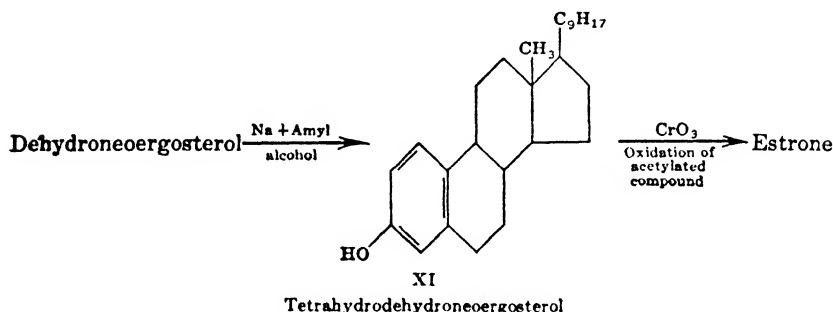
* Color reactions: Kober, *Biochem. Z.*, **239**, 209 (1931); Schwenk and Hildebrandt, *ibid.*, **259**, 240 (1933); Häussler, *Helv. Chim. Acta*, **17**, 531 (1934); Zimmermann, *Z. physiol. Chem.*, **233**, 257 (1935); Pincus, Wheeler, Young, and Zahl, *J. Biol. Chem.*, **116**, 253 (1936).
³⁷⁹ Doisy, Veler, and Thayer, *Am. J. Physiol.*, **90**, 329 (1929); *J. Biol. Chem.*, **88**, 499 (1930); **87**, 357 (1930).

³⁸⁰ Butenandt, *Naturwissenschaften*, **17**, 879 (1929); Butenandt and v. Ziegner, *Z. physiol. Chem.*, **188**, 1 (1930).

³⁸¹ Marrian, *Biochem. J.*, **24**, 435 (1930).

³⁸² Butenandt, *Z. physiol. Chem.*, **191**, 127, 140 (1930); Butenandt and Marrian, *ibid.*, **200**, 277 (1931); Butenandt and Störmer, *ibid.*, **208**, 129 (1932); Butenandt, Störmer and Westphal, *ibid.*, **208**, 149 (1932); Butenandt and Westphal, *ibid.*, **223**, 147 (1934); Butenandt and Thompson, *Ber.*, **67**, 140 (1934).

Doisy,³⁸³ and Marrian.³⁸⁴ Estrone is a phenolic ketone and estriol a phenolic glycol. Dehydration of the latter with potassium acid sulfate converts it into estrone.* Thus both compounds have the same nucleus. The ring system of the two hormones has been established by the following procedures: Estrone, when distilled with zinc, is dehydrogenated to chrysene (VII). The basic ring structure is not chrysene, however, for when estriol is fused with potassium hydroxide, ring D is opened to produce a dicarboxylic acid (VIII), which, by selenium dehydrogenation, is converted to a dimethylphenanthrol (IX); this, in turn, may be reduced by distillation over zinc to dimethylphenanthrene (X). The relationship to the sterols and bile acids is brought out in this transformation, for selenium dehydrogenation of etiobilanic acid (p. 1244) gives the same dimethylphenanthrene. The correlation with the sterols is neatly shown by a transformation due to Marker.³⁸⁵ When dehy-



droneoergosterol (p. 1288) is reduced with sodium and amyl alcohol, ring B is hydrogenated, giving tetrahydrodehydroneoergosterol (XI). Chromic acid acting on the acetyl derivative of this sterol oxidizes the side chain at C₁₇ to carbonyl, and on deacetylation, estrone is obtained.† Thus the ring nucleus and the positions of the functional groups are completely characterized.

³⁸³ Thayer, Levin, and Doisy, *J. Biol. Chem.*, **91**, 655 (1930); MacCorquodale, Thayer, and Doisy, *ibid.*, **99**, 327 (1933).

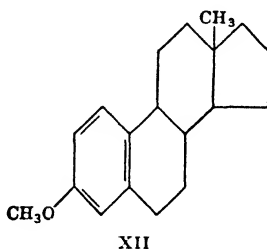
³⁸⁴ Marrian, *Biochem. J.*, **24**, 1021 (1930); Marrian and Haslewood, *ibid.*, **26**, 25, 1227 (1932).

* Butenandt in his earlier work obtained what he called β -follicular hormone from this fusion mixture. Apparently it was one of the polymorphic modifications of estrone. See reference 367.

³⁸⁵ Marker, Kammi, Oakwood, and Laucius, *J. Am. Chem. Soc.*, **58**, 1503 (1936). Fieser, Monograph, p. 219, suggested the possibilities of such a degradation.

† A serious objection to this work of Marker has been reported by Windaus and Deppe, [*Ber.*, **70**, 76 (1937)]. On reducing dehydroneoergosterol with sodium and alcohol, hydrogenation of ring A rather than of ring B takes place. It is possible that reduction of ring B occurs in Marker's transformation, since Remezov [*Rec. trav. chim.*, **55**, 797 (1936)] has

Prior to the work of Marker the positions of the functional groups and the other structural details had been established by satisfactory evidence. The phenolic hydroxyl group of estrone had been allocated to C₃ in the following ways: (1) The methyl ether of estrone was reduced to a desoxo compound (XII) and the product dehydrogenated in good yield (15 per cent) to a methoxy hydrocarbon; the structure of the latter as 7-methoxy-1,2-cyclopentenophenanthrene was established by synthesis.³⁸⁶ (2) By synthesis the dimethylphenanthrol (IX) obtained by the degradation of estriol was shown to have the structure 7-hydroxy-1,2-dimethylphenanthrene.³⁸⁷ The benzenoid character of ring A



was established by the phenolic properties,* by the absorption spectrum, and by the uptake of three moles of hydrogen on catalytic hydrogenation of desoxoestrone.

By a series of reactions that have a number of unusual features, Cohen, Cook, and Hewett³⁸⁸ have established the position of the carbonyl group and of the angular methyl group of estrone. The methyl ether of estrone was reacted with methylmagnesium iodide, the resulting carbinol was dehydrated, the unsaturated compound formed was reduced, and this reduction product was dehydrogenated with selenium. The final product proved to be 7-methoxy-3',3'-dimethyl-1,2-cyclopentenophenanthrene and not the expected 7-methoxy-3'-methyl-1,2-cyclopentenophenanthrene (cf. IV, p. 1231). The structure of the final hydrocarbon was established by synthesis. To explain its formation a

degraded neoergosterol to an estrogenic compound which apparently is isomeric with estrone, but in which ring B rather than ring A is aromatic. The estrogenic activity of the isomer is comparable to that of estrone.

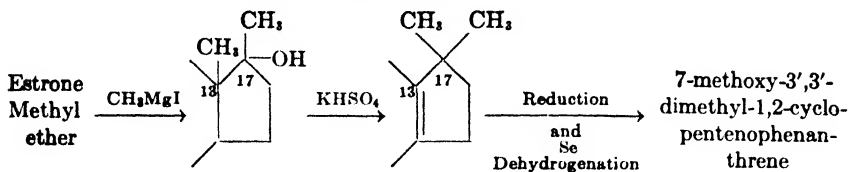
³⁸⁶ Cook and Girard, *Nature*, **133**, 377 (1934); Cohen, Cook, Hewett, and Girard, *J. Chem. Soc.*, 653 (1934).

³⁸⁷ Haworth and Sheldrick, *J. Chem. Soc.*, 864 (1934).

* For some reason as yet unexplained, estriol is more acidic than estrone: estriol, $K = 0.77 \times 10^{-9}$; estrone, $K = 0.44 \times 10^{-9}$ [Butenandt and Westphal, *Z. physiol. Chem.*, **223**, 147 (1934)].

³⁸⁸ Cohen, Cook, and Hewett, *J. Chem. Soc.*, 445 (1935).

molecular rearrangement must be assumed, and the course of the reaction may be schematically represented:



The carbinol formulated in this reaction has an hydroxyl group attached to a carbon adjacent to a quarternary carbon atom and is of the type in which dehydration should be accompanied by rearrangements. That the ring methyl group actually wanders to C₁₇ is shown by the fact that migration of the methyl group also occurs when the methyl ether of dihydroestrone is similarly dehydrated, reduced, and dehydrogenated with selenium. The product in this case is 7-methoxy-3'-methyl-1,2-cyclopentenophenanthrene, a hydrocarbon whose structure has also been established by synthesis.

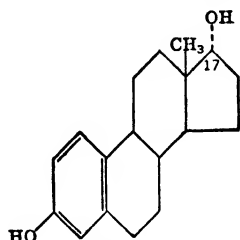
The methyl group that is present in the 3' position in the end products of these two transformations must have been originally attached to the ring system as an angular methyl group. The transformation shows that this group is attached at C₁₃ and that the carbonyl group of estrone is situated at C₁₇. No other conclusion is compatible with the evidence since the possibilities of rearrangements in the dehydrogenation with selenium are excluded by the reduction prior to this treatment.

The Estradiols. When estrone is reduced in alkaline media,* a mixture of α - and β -estradiol is formed.³⁸⁹ By fractional recrystallization from acetone and alcohol the mixture may be separated into α -estradiol (I), m.p. 176°, and β -estradiol (XIII), m.p. 223°; or, as Wintersteiner³⁸⁹ has shown, the α -compound may be removed by treatment in 80 per cent alcohol with digitonin with which it forms an

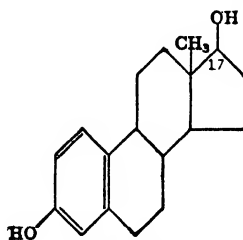
* Reduction of estrone in acid solution results principally in hydrogenation of ring A rather than of the C₁₇ carbonyl [Dirscherl, *Z. physiol. Chem.*, **239**, 53 (1936)]. In this hydrogenation new centers of asymmetry are created at C₃, C₆, and C₁₀, and thus eight hexahydrohormones and sixteen octahydrohormones are possible. Catalytic hydrogenation in alcohol in the presence of hydrochloric acid gives a mixture of stereoisomeric octahydroestrones of which the principal component is a product of m.p. 210–211°. Catalytic hydrogenation in acetic acid gives a mixture of hexahydroestrones, octahydroestrones, and hexahydrodesoxoestrones. One of the hexahydrohormones gives an insoluble digitonide, but, according to Dirscherl, the octahydroestrone, m.p. 210–211°, does not give an insoluble digitonide.

³⁸⁹ Schwenk and Hildebrandt, *Naturwissenschaften*, **21**, 177 (1933); Dirscherl, *Z. physiol. Chem.*, **239**, 53 (1936); Wintersteiner, *J. Am. Chem. Soc.*, **59**, 765 (1937); Whitman, Wintersteiner, and Schwenk, *J. Biol. Chem.*, **118**, 789 (1937); Butenandt and Goergens, *Z. physiol. Chem.*, **248**, 129 (1937).

insoluble precipitate. It seems probable that the C_{17} —OH of α -estradiol is *trans*, and of the β -estradiol *cis*, to the C_{13} — CH_3 . These configurations are shown in the structural formulas I and XIII. The physical



I α -Estradiol
(steric position of
 C_{17} —OH provisional)



XIII β -Estradiol
(steric position of
 C_{17} —OH provisional)

properties of the two compounds (Table XII) furnish the evidence for such configurations. By analogy to the stereoisomeric testosterones discussed later (p. 1377) a higher melting point and lower specific rotation is taken to indicate a *cis* configuration of the OH and CH_3 . Thus α -estradiol, which has a lower melting point and a higher specific rotation, is regarded as a compound in which the C_{17} —OH is *trans* to the angular methyl group.

α -Estradiol is the most potent estrogenic hormone known,* but β -estradiol is even less active than estrone.† Doisy and co-workers³⁹⁰ have shown that α -estradiol is present in the ovarian tissues and the follicular fluid and is therefore a true glandular sex hormone.‡ α -Estradiol is used in clinical medicine, since it is readily prepared from estrone and its potency is so much greater. The C_3 -benzoyl ester is generally employed in the therapeutic preparations as the effect is more prolonged than that of the unesterified hormones.§ Clinically the estrogenic hor-

* Even more potent than α -estradiol is a nitrogenous compound, $C_{20}H_{41}O_2N$, m. p. 95° , isolated from ovarian tissue by Andrews and Fenger, *Endocrinology*, **20**, 563 (1936). This compound gives a delayed but prolonged estrogenic response in a dosage of 10^{-3} g.

† A similar change in biological activity with spatial inversion has been noted in the synthetic plant hormone, α -(3-indole)-propionic acid. The *d*-acid has an activity of 48 billion oat units per gram while that of the *l*-acid is only 1.6 billion units per gram [Kögl, *Naturwissenschaften*, **25**, 465 (1937)].

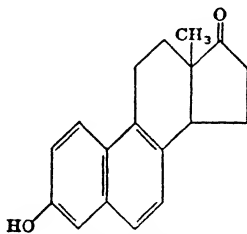
³⁹⁰ MacCorquodale, Thayer, and Doisy, *Proc. Soc. Exptl. Biol. Med.*, **32**, 1182 (1935); *J. Biol. Chem.*, **115**, 435 (1936).

‡ Prior to this Wintersteiner, Schwenk, and Whitman, *Proc. Soc. Exptl. Biol. Med.*, **32**, 1087 (1935), isolated α -estradiol from mares' urine. This, of course, did not establish the important fact that α -estradiol is the hormone produced in the ovary.

§ Esters of α -estradiol and estrone other than the benzoyl esters have been studied by Diracherl, *Z. physiol. Chem.*, **239**, 49 (1936), who examined a number of ester carbonates; and by Miescher and Scholz, *Helv. Chim. Acta*, **20**, 263 (1937), who studied a series of esters of the aliphatic acids. According to the latter authors some of the aliphatic esters are more potent than those previously described, but the details have not been published.

mones are finding their greatest use in alleviating the discomfort experienced at the menopause.

Estrogenic Hormones from Mares' Urine. In the urine of pregnant mares there are a number of highly unsaturated hormones in addition to estrone.³⁹¹ The compound known as equilenin is present in largest



XIV Equilenin

quantity, but, in addition, small amounts of hippulin, equilin, and at least one 17-dihydroequilenin³⁷⁴ have been found. Of these, equilenin (XIV) has been investigated most thoroughly. Its reactions indicate that it differs from estrone in that ring B as well as ring A is aromatic.* Equilin does not take up hydrogen in the presence of palladium, but is dehydrogenated to equilenin. This behavior has been interpreted by Dirscherl³⁹² as indicating a double bond at C₆:C₇, and by Cook³⁹³ as a double bond at C₈:C₉. Girard has made the interesting observation that in mares' urine during the course of pregnancy first estrone, then equilin, and finally equilenin are excreted in increasingly larger proportions.

Synthetic Estrogenic Compounds. A variety of synthetic estrogenic compounds have been discovered. Cook, Dodds, *et al.*,³⁹⁴ prompted by the observation that 1-keto-1,2,3,4-tetrahydrophenanthrene (XV) produces estrus, examined a number of related compounds. Especially potent were a number of diols derived from dibenzanthrene of the general formula shown (XVI). Of these the diethyl, di-*n*-propyl and

³⁹¹ Girard, *et al.*, *Compt. rend.*, **194**, 909, 1020 (1932); **195**, 981 (1932); Girard, Fridenson, and Sandulesco, *Compt. rend. soc. biol.* **112**, 964 (1933).

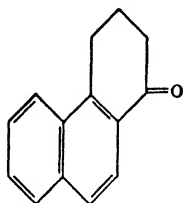
* The hydrogenation of equilenin proceeds in a manner analogous with that of estrone (note, p. 1363). Reduction with aluminum isopropoxide gives a mixture of C₁₇ isomers that can be separated by crystallization from alcohol. The more soluble dihydroequilenin melts at 215°, the less soluble at 248°. Attempts to reduce equilenin to dihydroequilenin with sodium give oily products in which hydrogenation of a naphthol ring has apparently occurred. Catalytic hydrogenation with the platinum catalyst of Adams results in the reduction of ring A and a loss of the C₁₃-OH [Marker, Kamm, Oakwood, and Tendick, *J. Am. Chem. Soc.*, **59**, 768 (1937)].

³⁹² Dirscherl and Hanusch, *Z. physiol. Chem.*, **233**, 13 (1935); **236**, 131 (1935).

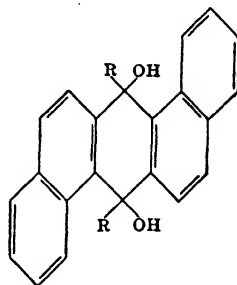
³⁹³ Cook and Roe, *J. Soc. Chem. Ind.*, **54**, 501 (1935).

³⁹⁴ Cook, Dodds, Hewett, and Lawson, *Proc. Roy. Soc. (London)*, **114B**, 272 (1934).

di-*n*-butyl derivatives are highly active, the greatest activity being shown by the di-*n*-propyl compound (0.05 mg. for estrus). In addition neoergosterol, calciferol, and ergosterol showed slight activity. A group of substituted phenanthrenes was also examined. Although most of the compounds were not estrogenic, 1,9-dimethylphenanthrene and the very active carcinogenic hydrocarbons, 1,2-benzpyrene and 5,6-cyclopenteno-1,2-benzanthrene produce estrus. In a later work³⁹⁵ the same authors



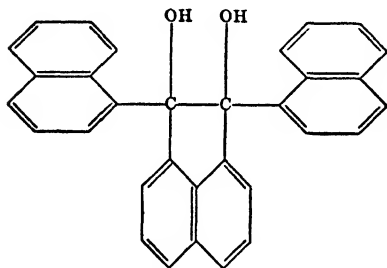
XV 1-Keto-1,2,3,4-tetrahydrophenanthrene



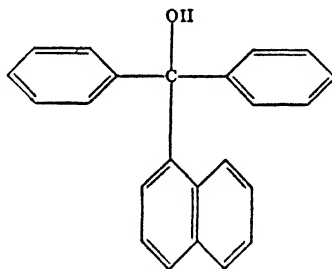
XVI Type formula of the 1,2,5,6-dibenzo-9,10-dialkylanthraquinols

showed that the synthetic estrogenic compounds bring about typical secondary female sex changes when injected in capons.

Dodds and co-workers³⁹⁶ investigated the problem of whether a phenanthrene nucleus is essential for estrogenic activity. The fact that



XVII 1,2-Dihydroxy-1,2-di- α -naphthylacenaphthene (active)



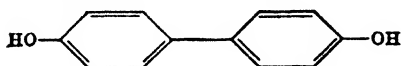
XVIII α -Naphthylidiphenylcarbinol (active)

calciferol produced estrus indicated that it was not, and following this clue a number of other substances were studied. In this investigation, 1,2-dihydroxy-1,2-di- α -naphthylacenaphthene (XVII) was the first active substance found in which the phenanthrene nucleus was absent.

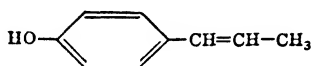
³⁹⁵ Cook, Dodds, and Greenwood, *ibid.*, **114B**, 286 (1934).

³⁹⁶ Dodds, *Helv. Chim. Acta*, **19**, E49 (1936); Dodds and Lawson, *Nature*, **139**, 627 (1937).

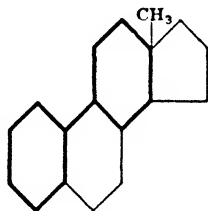
Simpler molecules were then studied with the result that triphenylcarbinol was found to be inactive, but α -naphthyldiphenylcarbinol (XVIII) was active. An even simpler molecule with estrogenic activity was 4,4'-dihydroxybiphenyl (XIX). None of these compounds, however, possessed activity comparable to that of the natural sex hormones. In the work with Lawson, Dodds found that many of the hydroxystilbenes were highly active and that the activity persisted over a long period of time. The most active substance of the series was *p*-hydroxypropenylbenzene (XX) which is found in nature as the methyl ether anethol. *p*-Hydroxypropenylbenzene was active in rats in a dose of 1 γ and therefore has the same potency as estrone.* In his earlier work Dodds offered the speculation that the activity of the estrogenic hormones may be due to the presence of a biphenyl type of structure. This is illustrated in formula XXI in which the biphenyl ring system is outlined in thick lines. In the light of the later work this speculation may have to be modified.



XIX 4,4'-Dihydroxybiphenyl
(active)



XX *p*-Hydroxypropenylbenzene
(activity equal to estrone)



XXI

These results raise the question of biological specificity. Is the estrous mechanism relatively unspecific? Apparently it is specific in the sense that a certain type of structure is essential. As Kögl³⁹⁷ has aptly expressed it, "in the case of the follicular hormone (oestrone) it has been found that the 'lock' can be opened not only by the classical 'key,' but, also, more or less easily, by rough copies, or even by skeleton keys."

The Corpus Luteum Hormone: Progesterone³⁹⁸

In the sexual cycle of the female the estrogenic hormones bring the animal into heat and, at the same time, an ovum begins to mature.

* Later work has shown that the activity is due to some polymer, or impurity, and not to *p*-hydroxypropenylbenzene itself [Dodds and Lawson, *Nature*, **139**, 1068 (1937)].

³⁹⁷ Kögl, *Brit. Assoc. Advancement Sci. Repts.* (Leicester), 600 (1933).

³⁹⁸ Reviews: Allen, *Science*, **83**, 89 (1935); Westphal, *Ergob. Physiol.*, **37**, 273 (1935); Hohlweg and Schmidt, *Klin. Wochschr.*, **15**, 265 (1936).

At the time when the ovum descends into the uterine passages, the corpus luteum, a small yellow body in the ovary, begins to produce the hormone progesterone. Just as the estrogenic hormones bring about a condition favorable to mating in the female, so does the corpus luteum hormone by causing a proliferation of the endometrium (the lining of the uterus) create a condition ideal for the implantation of the fertilized ovum. If implantation occurs, the corpus luteum persists and continues to furnish its hormone during most of the remainder of pregnancy in the majority of species. In the absence of implantation the corpus luteum degenerates and the animal once more comes into estrus or lapses into anestrus, depending on the nature of her cycle. So, through the complementary action of the estrogenic hormones and the corpus luteum hormone, the sexual cycle in females is controlled.

The isolation of progesterone * was made possible by Corner and W. M. Allen³⁹⁹ through the development of a biological test to measure its activity. In this test a female rabbit is castrated a day after mating and an oil solution of the hormone or hormonal preparation is injected subcutaneously each day for five days. A total of 1 mg. (1 rabbit unit) of pure progesterone brings the endometrium into complete proliferation which is easily recognized in a histological section from the uterus of the animal on the sixth day. Clauberg⁴⁰⁰ has developed a modification of this test by using immature rabbits and preparing them for the assay by maturing the uterus through injection of estrogenic hormones. Progesterone has no effect on the uterus of immature rabbits.

Isolation of Progesterone. Following the development of the Allen-Corner test, Allen⁴⁰¹ isolated potent fractions from the corpus luteum of pregnant sows, but the isolation of the pure hormone was first announced by Butenandt.⁴⁰² Shortly after this Slotta *et al.*,⁴⁰³ Allen and Wintersteiner,⁴⁰⁴ and Hartmann and Wettstein⁴⁰⁵ also described

* The name progesterone was adopted by international agreement [Allen, Butenandt, Corner, and Slotta, *Science*, **82**, 153 (1935); *Ber.*, **68**, 1746 (1935); *Nature*, **136**, 303 (1935)]. Prior to this it was known as progestin, the corpus luteum hormone, or luteosterone.

³⁹⁹ Corner and Allen, *Am. J. Physiol.*, **86**, 74 (1928); **88**, 326 (1929); Allen and Corner, *ibid.*, **88**, 340 (1929).

⁴⁰⁰ See Clauberg, "Die weibliche Sexualhormone," Springer, Berlin (1933); Butenandt, Westphal, and Hohlweg, *Z. physiol. Chem.*, **227**, 84 (1934).

⁴⁰¹ Allen, *J. Biol. Chem.*, **98**, 591 (1932).

⁴⁰² Butenandt, *Verhandl. deut. Ges. inn. Med.* (April, 1934); Butenandt and Westphal, *Ber.*, **67**, 1440 (1934); Butenandt, Westphal, and Hohlweg, *Z. physiol. Chem.*, **227**, 84 (1934).

⁴⁰³ Slotta, Ruschig, and Fels, *Ber.*, **67**, 1624 (1934); cf. Neuhaus, *Ber.*, **67**, 1627 (1934).

⁴⁰⁴ Allen and Wintersteiner, *Science*, **80**, 190 (1934); Wintersteiner and Allen, *J. Biol. Chem.*, **107**, 321 (1934).

⁴⁰⁵ Hartmann and Wettstein, *Helv. Chim. Acta*, **17**, 878, 1365 (1934).

pure preparations. The hormone occurs in two crystalline modifications: α -progesterone, m.p. 128.5° (prisms), and β -progesterone, m.p. 121° (needles), both of the same physiological potency. The methods of isolating this hormone have been described by the several groups of investigators, but the best process appears to be that recently described by Allen.⁴⁰⁶

The Structure of Progesterone. The hormone progesterone has the composition $C_{21}H_{30}O_2$ and is a diketone with strong absorption at 240 $m\mu$ due to the grouping $C=C-C=O$. The structure of the hormone was first suggested by Slotta,⁴⁰⁷ but the confirmation of this structure is due largely to the synthetic work of Butenandt. In the first of the two methods that have been developed for the preparation of the hormone, stigmasterol (p. 1283) is converted through the acetate of β -3-hydroxy- Δ^5 -bisorcholeonic acid (XXII) to the unsaturated Δ^5 -pregnenolone [3(*cis*)-hydroxy-20-keto-5-pregnene, XXIII]. Direct oxidation⁴⁰⁸ of this unsaturated ketone gives a poor yield of a mixture with progestational activity.* By a better procedure⁴⁰⁹ the unsaturated hydroxy ketone is converted to the dibromo compound, oxidized to a dibromo diketone, and finally debrominated by zinc to progesterone (cf. conversion of cholesterol to cholestenone, p. 1239). In the second method pregnanediol (XXIV) (p. 1282) is oxidized to pregnanedione, and a double bond introduced by bromination (at C_4) and removal of hydrogen bromide from XXV by treatment with pyridine.⁴¹⁰

Allopregnanolone. From the extracts which yield progesterone an inactive compound, *allopregnanolone*, has been isolated.⁴¹¹ The structure of the compound has been established as 3(*cis*)-hydroxy-20-keto*allo*pregnane by synthesis from β -3-hydroxybisnor*allo*choleonic acid, one of the products of the degradation of stigmasterol.⁴¹² The *cis* position of the C_3-OH group to the $C_{10}-CH_3$ is shown by the mode of synthesis and by the formation of an insoluble digitonide. On oxidation *allopregnanolone* is converted to *allopregnanedione*.

The $C_{17}-CO-CH_3$ group of *allopregnanolone* or *pregnenolone*

⁴⁰⁶ Allen and Goetsch, *J. Biol. Chem.*, **116**, 653 (1936); cf. Butenandt and Westphal, *Ber.*, **69**, 443 (1936).

⁴⁰⁷ Slotta, Ruschig, and Fels, *Klin. Wochschr.*, **13**, 1207 (1934).

⁴⁰⁸ Butenandt, Westphal, and Cobler, *Ber.*, **67**, 1611 (1934).

* With Oppenauer's reagent (footnote, p. 1239) the transformation of Δ^5 -pregnenolone to progesterone is very satisfactory [Oppenauer, *Rec. trav. chim.*, **56**, 137 (1937)].

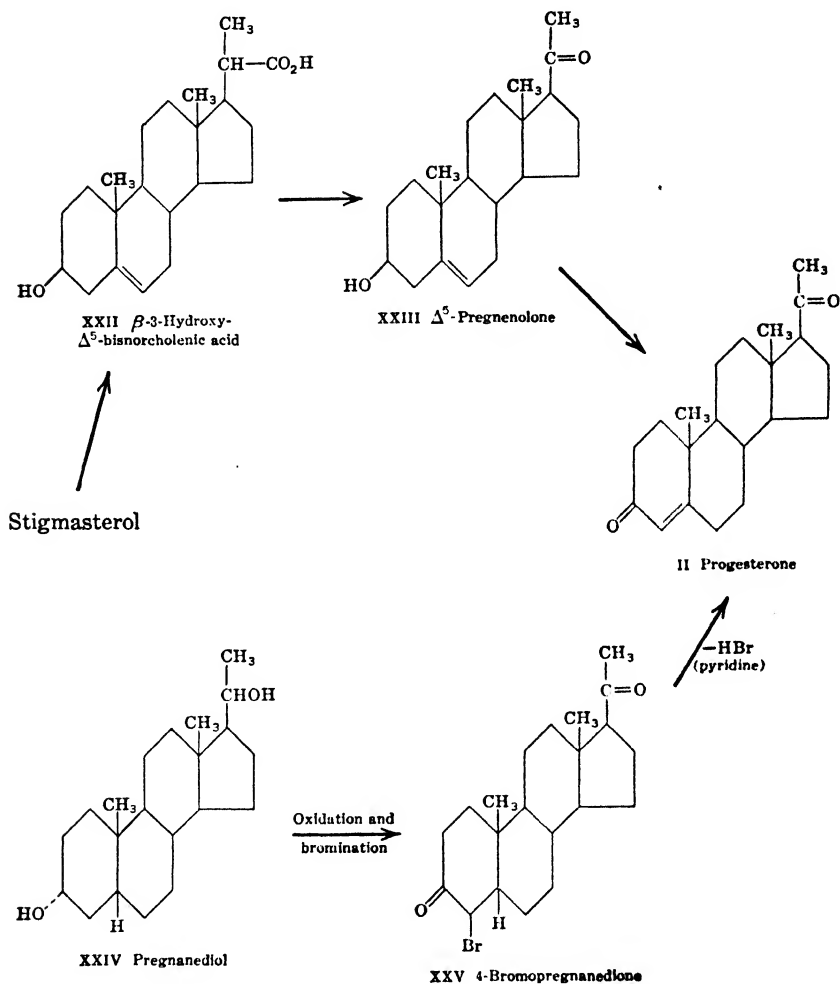
⁴⁰⁹ Butenandt and Schmidt, *Ber.*, **67**, 1901 (1934); Butenandt and Westphal, *Ber.*, **67**, 2085 (1934); Fernholz, *Ber.*, **67**, 1855, 2027 (1934).

⁴¹⁰ Butenandt and Schmidt, reference 409; see also Butenandt and Schmidt, *Ber.*, **67**, 2088 (1934).

⁴¹¹ Butenandt and Mamoli, *Ber.*, **67**, 1897 (1934).

⁴¹² Fernholz, *Z. physiol. Chem.*, **230**, 185 (1934).

undergoes a rearrangement to the extent of about 30 per cent when these compounds are refluxed in a solution of 5 per cent methanolic alkali.⁴¹³ Although it is improbable that this treatment affects the



position of the C_3 —OH group, the resulting iso compounds do not give insoluble digonides. Since the iso compounds have optical properties quite different from those of their parent compounds, the transformation may be followed by measuring the optical rotation. To illus-

⁴¹³ Butenandt and Mamoli, *Ber.*, **68**, 1847 (1935); Butenandt and Fleischer, *Ber.*, **70**, 96 (1937).

trate this the melting point and specific rotations of the pairs from *allopregnanolone*, *pregnenolone*, and *allopregnanedione* are given below:

<i>Allopregnanolone</i> m.p. 194.5° [α] _D : + 90.8°	<i>Allopregnanedione</i> m.p. 200.5° [α] _D : + 126.9°	Δ^5 - <i>Pregnenolone</i> m.p. 190° [α] _D : + 28.2°
<i>Isoallopregnanolone</i> m.p. 147-148° [α] _D : + 6.1°	<i>Isoallopregnanedione</i> m.p. 134-135° [α] _D : - 14.6°	Δ^5 - <i>Isopregnenolone</i> m.p. 172-173° [α] _D : - 126°

Allopregnanolone may be a stage of the *in vivo* reduction of progesterone or may be the product of an independent biosynthesis. As pointed out earlier (p. 1282), the two compounds pregnanediol and *allopregnanediol* probably originate from progesterone.* It is noteworthy that the corpus luteum hormone is excreted in the reduced form, while the estrogens appear in the oxidized state.

Specificity. Progesterone has the highest specificity of the sex hormones. Since hydrogenation of the sex hormones often results in increase in activity, dihydroprogesterone, 3-keto-20-hydroxy-5-pregnene, might be expected to be especially potent, but this compound is inactive in doses of 1 mg. Other substances have been tested in which the carbonyl at C₃ and a double bond at C₄ are present, but nearly all have been found to be inactive.* The isomers of progesterone with unsaturation at C₁:C₂ or C₅:C₆ are also inactive,⁴¹⁴ but the 3-enol acetate of progesterone (enolization from C₂) has been reported to have an activity comparable to that of the pure hormone.⁴¹⁵ In addition to the enol acetate, the male hormones testosterone and 17-methyltestosterone (p. 1382) have progestational activity but in a much lower degree.

The Androgenic Hormones⁴¹⁶

From the urine of both males and females and from testicular extracts, a number of hormones have been isolated which, when injected into castrate or immature males, bring about the restoration or development, respectively, of the latent secondary sexual characteristics. Like

* Human pregnancy urine contains 1-2 mg. per liter of *epiallopregnanolone*, the stereoisomer at C₃ of *allopregnanolone*. In contrast to *allopregnanolone* which is inactive, *epiallopregnanolone* has male hormone activity. [Marker, Kamm, and McGrew, *J. Am. Chem. Soc.*, **59**, 616 (1937); Marker, Kamm, Jones, Wittle, Oakwood, and Crooks, *ibid.*, **59**, 768 (1937)].

* For list see Westphal, reference 398.

⁴¹⁴ Butenandt and Mamoli, *Ber.*, **68**, 1850 (1935); Butenandt and Schmidt-Thomé, *Ber.*, **69**, 882 (1936).

⁴¹⁵ Westphal, *Naturwissenschaften*, **24**, 696 (1936).

⁴¹⁶ Reviews: Dannenbaum, *Ergeb. Physiol.*, **38**, 796 (1936).

the female sex hormones, it was essential to have a biological method of assay for the study of these male hormones. The first feasible method was one developed by Gallagher and Koch⁴¹⁷ in which the effect of these hormones on comb growth in capons is measured. Numerous modifications of the original test have been developed. Gallagher and Koch originally proposed that one comb or capon unit be taken as that amount of the hormone necessary to produce a 20 per cent increase in comb area with carefully standardized leghorn capons. At the present time, the unit of activity (international comb unit) has been defined by international agreement as 0.1 mg. (100 γ) of the male hormone androsterone (Gr., *andro*, male).³⁶⁸ The assay is carried out in two groups of standardized capons, one with the standard hormone, the other with the substance under investigation. The growth of the comb is determined by means of a shadowgraph or by direct measurement of certain portions of the comb.⁴¹⁷

In a second method of assay, the effect on the accessory sex organs in castrate and immature rats (or mice) is measured. The assay is not as well defined as that on capons, and in this discussion only the values obtained by the methods of Butenandt and Tscherning⁴¹⁸ and of Tschopp⁴¹⁹ will be cited.* In the Butenandt-Tscherning method immature rats (4 weeks old) are injected with a solution of the hormone in sesame oil for eight successive days, and on the ninth day, a histological examination of the seminal vesicles is made. By varying the level at which the hormone is injected, that concentration which brings about development of the seminal vesicles comparable to that of control rats is determined. In the test of Tschopp, rats are castrated while immature, and after thirty days are injected subcutaneously once a day for ten days with an oil solution of the hormone. At the end of the ten-day period, the animal is killed and the seminal vesicles and the prostate weighed. The daily level of hormone necessary for the development of a seminal vesicle weighing 40 mg. is determined either directly or by extrapolation. The results in both cases are expressed as rat units (R.U.), but the values from one assay are only qualitatively comparable to those of the other.

Isolation of the Androgenic Hormones. In 1931-1932 Butenandt⁴²⁰ reported the isolation of three compounds from the oil obtained by the

⁴¹⁷ Gallagher and Koch, *J. Pharmacol.*, **40**, 327 (1930). For review of the test, see Greenwood, Blyth, and Callow, *Biochem. J.*, **29**, 1400 (1935).

⁴¹⁸ Tscherning, *Ber.*, **68**, 679 (1935).

⁴¹⁹ Tschopp, *Arch. intern. pharmacodynamie*, **52**, 381 (1936).

* For other methods see Dannenbaum, reference 416.

⁴²⁰ Butenandt, *Z. angew. Chem.*, **44**, 905 (1931); *ibid.*, **45**, 655 (1932); *Nature*, **130**, 238 (1932).

extraction of male urine which had previously been boiled with hydrochloric acid.* The substance obtained in largest quantity (20–25 mg.) was a hydroxyketone, $C_{19}H_{30}O_2$, to which the name androsterone was given. Other substances present were dehydroandrosterone, $C_{19}H_{26}O_2$, and a chloroketone, $C_{19}H_{27}OCl$, evidently formed from dehydroandrosterone by replacement of the hydroxyl by chlorine.† In analogy to the estrogenic hormones, a structure was suggested for androsterone that was essentially correct but did not show the spatial configuration of the molecule. Plausibility was given to this suggestion by the discovery that a completely reduced estrone, octahydroestrone, had male hormone activity.⁴²¹ At this point the structure suggested by Butenandt and its spatial chemistry were definitely established by Ruzicka⁴²² by oxidizing the acetates of dihydrocholesterol and *epidi*hydrocholesterol (XXVI) to the corresponding hydroxyetioallocholanones. Both these compounds were physiologically active, 3(*trans*)-hydroxy-17-ketoetioallocholanone (XXVII) from *epidi*hydrocholesterol being identical with natural androsterone. The epimer isoandrosterone, 3(*cis*)-hydroxy-17-ketoetioallocholanone, is about one-seventh as potent. The corresponding etiocholanones from coprosterol and *epi*coprosterol are inactive even in doses fourteen times that of androsterone. Shortly after this work of Ruzicka, Butenandt⁴²³ published the results of his examination of androsterone isolated from urine and fully confirmed the structure XXVII.

The structure of the male hormone dehydroandrosterone (XXIX) [3(*cis*)-hydroxy-17-keto-5-etioallocholanone] ‡ was easily elucidated by a

*A fourth, but inactive, compound, $C_{19}H_{32}O_2$, m. p. 232° , $[\alpha]_D + 29$ (alc.) was also obtained. This has now been characterized as *epi*etiocholan-3,17-diol [3(*trans*), 17-dihydroxyetiocholanone] by Ruzicka, Goldberg, and Bosshard, *Helv. Chim. Acta*, **50**, 541 (1937), and by Butenandt, Tscherning, and Dannenberg, *Z. physiol. Chem.*, **248**, 205 (1937), and is the only compound of the coprosterane type that has been isolated from the urine. It is uncertain, however, whether the compound is actually present in the urine or is formed from some other compound during isolation.

† Subsequently two methods have been developed for the conversion of dehydroandrosterone to the chloro ketone. By the first of these dehydroandrosterone is converted to the methyl ether through the stage of the *p*-toluenesulfonic ester, and the ether converted to the chloride by treatment with concentrated hydrochloric acid [Butenandt and Grosse, *Ber.*, **69**, 2776 (1936)]. By the second method dehydroandrosterone is treated with phosphorus pentachloride in chloroform [Wallis and Fernholz, *J. Am. Chem. Soc.*, **59**, 764 (1937)]. Good yields are obtained by both methods.

⁴²¹ Schoeller, Schwenk, and Hildebrandt, *Naturwissenschaften*, **21**, 286 (1933); Dirscherl and Voss, *ibid.*, **22**, 315 (1934).

⁴²² Ruzicka, Goldberg, and Brüngger, *Helv. Chim. Acta*, **17**, 1389 (1934); Ruzicka, Goldberg, Meyer, Brüngger, and Eichenberger, *ibid.*, **17**, 1395 (1934); Ruzicka, Goldberg, and Wirz, *ibid.*, **18**, 61 (1935).

⁴²³ Butenandt and Tscherning, *Z. physiol. Chem.*, **229**, 167, 185 (1934).

‡ Ruzicka calls this compound *trans*-dehydroandrosterone; Fieser, dehydroisoandrosterone. Most of the other workers refer to it by the simple name dehydroandrosterone.

further application of the chromic oxide oxidation method. At nearly the same time Butenandt,⁴²⁴ Ruzicka,⁴²⁵ and Wallis and Fernholz⁴²⁶ reported on the preparation of this compound by chromic acid oxidation of acetylcholesterol dibromide followed by debromination with zinc, but priority belongs to Schoeller.⁴²⁷

The Testicular Hormones. In the summer of 1935 the group of workers headed by Laqueur⁴²⁸ presented convincing evidence that the male hormone obtained from testicles was different from those that had been obtained from the urine. This was apparent from the greater potency and from the fact that the potency of extracts was destroyed by treatment with alkali. A few milligrams of this very potent compound was isolated and given the name testosterone. The testicular hormone was a diketone with strong absorption at 240 m μ , and from the fact that extracts containing progesterone are also inactivated by alkali, it seemed probable that testosterone was structurally similar to progesterone. This speculation was almost immediately confirmed.

The structure of testosterone (III) was established by David⁴²⁹ of Laqueur's group through its oxidation to Δ^4 -androstenedione (XXX), a compound which had previously been prepared from dehydroandrosterone. This structure was confirmed by the syntheses developed independently by Butenandt⁴³⁰ and Ruzicka⁴³¹ at nearly the same time; the transformation shown (p. 1375) is due to Ruzicka. By this method the acetate of dehydroandrosterone is reduced in the presence of nickel to the acetate of Δ^5 -androstenediol. The product is benzoylated at the C₁₇—OH and saponified at ca. 15°. In saponification hydrolysis takes place preferentially at C₃ to give a C₁₇ half ester which can be converted to the ester of the α , β -unsaturated ketone either by direct oxidation with chromic acid or by oxidation of the 5,6-dibromo compound followed by debromination with zinc. The method of Butenandt is similar to that of Ruzicka, save that the diacetate rather than the mixed ester of Δ^5 -androstenediol is one of the intermediate products.

A second male hormone, m.p. 129–130°, has been isolated from

⁴²⁴ Butenandt, Dannenbaum, Hanisch, and Kudzus, *ibid.*, **237**, 57 (1935).

⁴²⁵ Ruzicka and Wettstein, *Helv. Chim. Acta*, **18**, 986 (1935).

⁴²⁶ Wallis and Fernholz, *J. Am. Chem. Soc.*, **57**, 1379, 1504 (1935).

⁴²⁷ Schoeller, Serini, and Gehrke, *Naturwissenschaften*, **23**, 337 (1935).

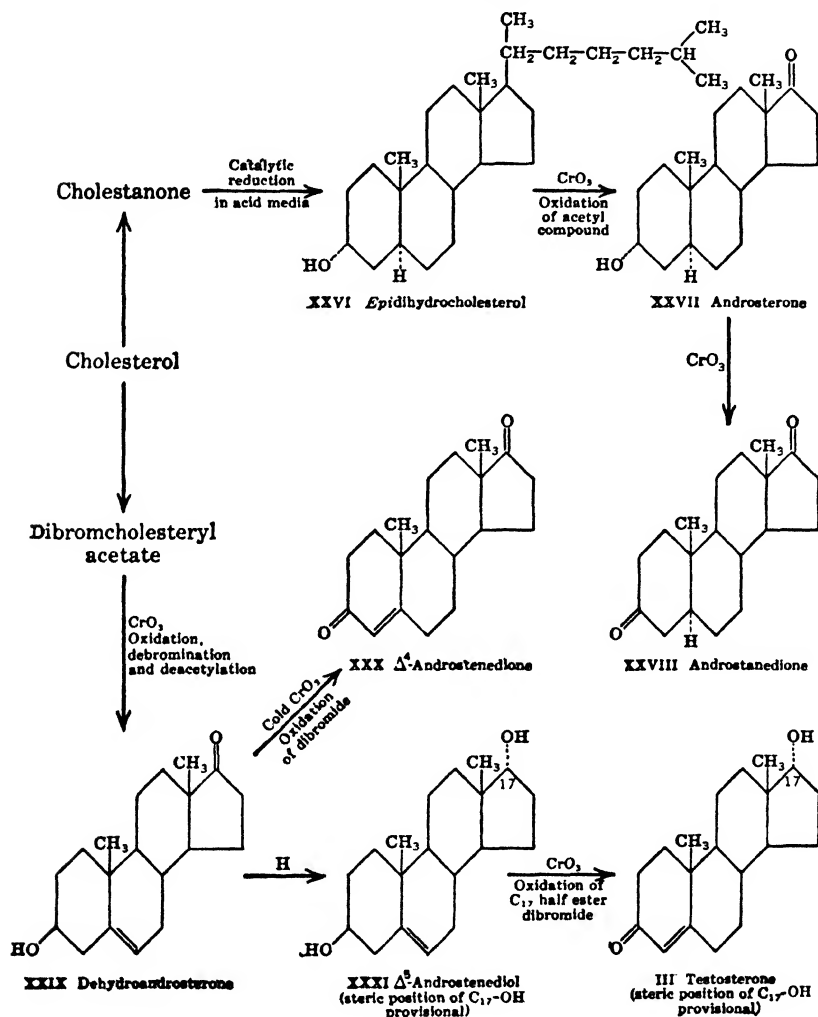
⁴²⁸ David, Dingemanse, Freud, and Laqueur, *Z. physiol. Chem.*, **233**, 281 (1935).

⁴²⁹ David, *Acta Brevi-Neerland. Physiol. Pharmacol. Microbiol.*, **5**, 85, 108 (1935).

⁴³⁰ Butenandt and Hanisch, *Ber.*, **68**, 1859 (1935); *Z. physiol. Chem.*, **237**, 89 (1935).

⁴³¹ Ruzicka and Wettstein, *Helv. Chim. Acta*, **18**, 1264 (1935); Ruzicka, Wettstein, and Kagi, *ibid.*, **18**, 1478 (1935).

testicular extracts by Ogata and Hirano.⁴³² At one time this compound was thought by Ruzicka⁴³² to be androstenedione, m.p. 132–133°, but



Hirano⁴³³ has shown that it is different. In any event there are at least two testicular hormones and possibly more.*

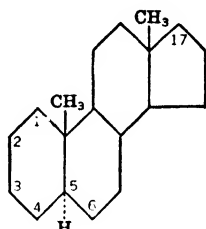
⁴³² Ogata and Hirano, *J. Pharm. Soc. Japan*, **54**, 199 (1934) [*C.A.*, **29**, 1871 (1935)]; Ruzicka and Wettstein, *Helv. Chim. Acta*, **18**, 1264 (1935).

⁴³³ Hirano, *J. Pharm. Soc. Japan*, **56**, 122 (1936); cited by Dannenbaum, *Ergeb. Physiol.*, **35**, 801 (footnote) (1936).

* From hog testes Hirano, *J. Pharm. Soc. Japan*, **56**, 717 (1936) [*C.A.*, **31**, 3125 (1937)], has isolated small amounts of four inert substances, A, B, C, and D. Compound A (testa-

From the urine of pregnant women a compound with male hormone activity has been isolated by Marker⁴³⁴ and has been shown to have the structure *epiallopregnanolone* [3(*trans*)-hydroxy-17-acetyl-etioallocholane]. This substance is regarded as an intermediary product in the biochemical reduction of progesterone to *allopregnanediol*.* *Epiallopregnanolone* is devoid of female hormone activity, but has male hormone activity comparable to that of androsterone.

The physical properties and physiological potencies of these natural and the related "artificial" sex hormones are shown in Table XIII. The data are taken from the several reviews, and from summaries by Deanesly and Parkes⁴³⁵ and by Ruzicka.⁴³⁶ The compounds are all named as derivatives of the parent hydrocarbon *etioallocholane* (androsterane) (XXXII). This hydrocarbon was not known prior to the work on the androgenic hormones and since then it has been pre-



XXXII Etioallocholane
(Androstane)

pared by the energetic Clemmensen reduction of androstanedione (XXVIII).⁴³⁷ Androstane melts at 49–50° as against 79–80° for the isomeric etiocholane.

lolone), $C_{21}H_{32}O_3$ (?), m.p. 258–264°, is a hydroxy diketone and forms an insoluble diglucuronide. The ring system is saturated and the compound reduces aldehyde reagents. Hirano has suggested that testalolone is 3-hydroxy-17-glyoxyletioallocholane. Compound B appears to be the monopalmitate of propane-1,2-diol. Compound C, $C_{19}H_{40}O_3$ (?), m.p. 65–66°, has been named testriol and is thought to be an open chain trihydroxy alcohol. Compound D, m.p. 219–224°, is a compound containing 23 or 24 carbon atoms and 3 atoms of oxygen.

⁴³⁴ Marker, Kamm, and McGrew, *J. Am. Chem. Soc.*, **59**, 616 (1932); Marker, Kamm, Jones, Wittle, Oakwood, and Crooks, *ibid.*, **59**, 768 (1937).

* A complete structural correlation of the female sex hormone progesterone and the male sex hormone androsterone has been established by the conversion of *allopregnanediol* to androstanedione. [Marker, Kamm, Jones, and Oakwood, *J. Am. Chem. Soc.*, **59**, 614 (1937).] The steps in the conversion are: Oxidation of *allopregnanediol* to *allopregnanolone*, dehydration at C_{20} by means of zinc chloride, and cleavage of the double bond at C_{17} and C_{20} by treatment with ozone.

⁴³⁵ Deanesly and Parkes, *Biochem. J.*, **30**, 291 (1936).

⁴³⁶ Ruzicka, Goldberg, and Rosenberg, *Helv. Chim. Acta*, **18**, 1487 (1935).

⁴³⁷ Butenandt and Tscherning, *Z. physiol. Chem.*, **229**, 185 (1934); Reichstein, *Helv. Chim. Acta*, **19**, 979 (1936).

The Stereochemistry of the Hydroxyl Groups. The difference in the ease of saponification of the ester groupings at C₃ and C₁₇ in Δ^5 -androstenediol has been interpreted by Ruzicka⁴³⁸ as indicating opposite spatial configuration of these groups with reference to the positions C₅ and C₁₃, respectively. The work of Vavon and Jakubowicz⁴³⁹ forms the basis for this view. These investigators found that the esters of *epidihydrocholesterol* are saponified more easily than the corresponding esters of *dihydrocholesterol*; thus an acetoxy group *cis* to the C₅—H is hydrolyzed more easily than one *trans* to this position. Since the acetoxy group at C₁₇ in Δ^5 -androstenediol diacetate is hydrolyzed less easily than the one at C₃, it follows that the C₁₇—OH of Δ^5 -androstenediol is *trans* to the C₁₃—CH₃ if the same principles apply to this position as to C₃. The results of catalytic hydrogenation confirm this view since the conditions under which the hydrogenation is carried out (alcoholic solution) are favorable to the formation of a *trans* configuration (v. Auwers-Skita rule). Ruzicka⁴⁴⁰ was able to isolate from the mother liquors of large-scale preparation of Δ^5 -androstenediol a compound with different physical and physiological properties, and to this compound (*cis*- Δ^5 -androstenediol) he has assigned a *cis* configuration of the C₁₇—OH group. From *cis*- Δ^5 -androstenediol a *cis*-testosterone was prepared. As the data of Table XIII show, the physiological potency of these compounds with a *cis* configuration of the C₁₇—OH group is much lower than the corresponding *trans* compounds.

The stereochemistry is further illustrated by the four theoretically possible androstane diols shown in structures XXXIII-XXXVI, of which three are known. Not all the names given here to these compounds are official but are merely offered as a means of differentiation among the compounds in connection with their preparation which is described below. Naturally there is a certain element of doubt as to the correctness of the structures which have been assigned.*

Androgenic Hormones from Androsterone. After the structures of the natural hormones had been determined, both Butenandt and Ruzicka studied the transformations in structure that could be effected by synthetic methods. Most of the transformations begin with androsterone, isoandrosterone, or dehydroandrosterone. Urine is not a satisfactory source of either androsterone or dehydroandrosterone, since the content of androsterone in urine of both males and females is

⁴³⁸ Ruzicka and Goldberg, *ibid.*, **19**, 99 (1936); Ruzicka and Kägi, *ibid.*, **19**, 842 (1936).

⁴³⁹ Vavon and Jakubowicz, *Bull. soc. chim.*, **53**, 581 (1933).

⁴⁴⁰ Ruzicka and Kägi, *Helv. Chim. Acta*, **19**, 842 (1936).

* An example may be cited to show the uncertainty. The diacetate of isoandrostanediol is hydrolyzed more easily at C₃ than at C₁₇—Butenandt and Dannenberg, *Ber.*, **69**, 1158 (1936)—but according to the views of Ruzicka this should not be the case.

TABLE XIII
ANDROGENIC HORMONES AND RELATED COMPOUNDS *

Hormone	Structural Modification of Etioallocholan †	Formula	M. P., ° C.	[α] _D (alcohol)	Physiological Potency		
					Gammas per I. C. U. ‡	Gammas per R. U. §	Buten- Tschopp and t
<i>Diketones</i>							
Δ ¹ -Androstenedione	3,17-Diketo-Δ ¹	C ₁₉ H ₂₆ O ₂	139-140	+ 6.8	inact.
Δ ⁴ -Androstenedione	3,17-Diketo-Δ ⁴	C ₁₉ H ₂₆ O ₂	169	+185	120	78	500
Δ ⁵ -Androstenedione	3,17-Diketo-Δ ⁵	C ₁₉ H ₂₆ O ₂	158	> ca. 250	1500
Androstanedione	3,17-Diketo-	C ₁₉ H ₂₈ O ₂	132-133 c.	+111	100-120	300	700
<i>Ketone Alcohols</i>							
Dehydroandrosterone	3(<i>cis</i>)-Hydroxy-17-keto-Δ ⁵	C ₁₉ H ₂₆ O ₂	140-141 c. ¶ 150-152 c. **	+ 11	200-300	3000
<i>epi</i> -Dehydroandrosterone	3(<i>trans</i>)-Hydroxy-17-keto-Δ ⁵	C ₁₉ H ₂₆ O ₂	221	0	100	ca. 500
Testosterone	3-Keto-17(<i>trans</i>)-hydroxy-Δ ⁴	C ₁₉ H ₂₆ O ₂	154-154.5 c.	+109	13-17	16	100
<i>cis</i> -Testosterone	3-Keto-17(<i>cis</i>)-hydroxy-Δ ⁴	C ₁₉ H ₂₆ O ₂	220-221	+ 71.5	400	>1000	

Dihydrotestosterone.....	3-Keto-17 (<i>trans</i>)-hydroxy- 3 (<i>trans</i>)-Hydroxy-17-keto-	$C_{19}H_{30}O_2$	180-181 c.	+ 32.4	25-30	50
Androsterone.....	3 (<i>cis</i>)-Hydroxy-17-keto-	$C_{19}H_{30}O_2$	184-185 c.	+ 96	100	325	1000
Isoandrosterone.....	3 (<i>cis</i>)-Hydroxy-17-keto-	$C_{19}H_{30}O_2$	170-171	+ 88	700-850	5000
6-Oxotestosterone.....	3,6-Diketo-17-hydroxy- Δ^4 -	$C_{19}H_{28}O_3$	203-205	-58 (acetone)	11
Testosterone acetate.....	3-Keto-17-acetoxy- Δ^4 -	$C_{21}H_{32}O_3$	138	+ 87.7	13	50
Δ^5 -Testosterone acetate.....	3-Keto-17-acetoxy- Δ^5 -	$C_{21}H_{30}O_3$	130-147	- 30.6	ca. 50	50
<i>E</i> piallopregnanolone.....	3 (<i>trans</i>)-Hydroxy-17-acetyl-	$C_{31}H_{54}O_2$	170	ca. 100

Dialcohols

Δ^4 -Androstenediol.....	3 (<i>cis</i>), 17 (<i>trans</i>)-Dihydroxy- Δ^4 -	$C_{19}H_{30}O_2$	155	200
Δ^5 -Androstenediol.....	3 (<i>cis</i>), 17 (<i>trans</i>)-Dihydroxy- Δ^5 -	$C_{19}H_{30}O_2$	182 c.	- 55.5	500	750
<i>cis</i> - Δ^5 -Androstenediol.....	3 (<i>cis</i>), 17 (<i>cis</i>)-Dihydroxy- Δ^5 -	$C_{19}H_{30}O_2$	198-198.5	850-1000
Androstenediol.....	3 (<i>trans</i>), 17 (<i>trans</i>)-Dihydroxy-	$C_{19}H_{32}O_2$	223 c.	+ 12.6	23-33	95	300
<i>cis</i> -Androstenediol.....	3 (<i>cis</i>), 17 (<i>cis</i>)-Dihydroxy-	$C_{19}H_{32}O_2$	213, 5-214.4 c.
Isoandrostenediol.....	3 (<i>cis</i>), 17 (<i>trans</i>)-Dihydroxy-	$C_{19}H_{32}O_2$	108 c.	+ 4.2	520-600	750

Methyl Derivatives

Methyltestosterone.....	3-Keto-17-hydroxy-17-methyl- Δ^4 -	$C_{20}H_{30}O_2$	163-164	25-80
Methyldihydrotestosterone.....	3-Keto-17-hydroxy-17-methyl-	$C_{20}H_{32}O_2$	192-193 c.	15-24	22
17-Methylandrostanediol.....	3 (<i>trans</i>), 17-Dihydroxy-17-methyl-	$C_{20}H_{34}O_2$	184-185 c.	ca. 33
17-Methylisoandrostanediol.....	3 (<i>cis</i>), 17-Dihydroxy-17-methyl-	$C_{20}H_{34}O_2$	210-211 c.	ca. 400

* Data from Dannenbaum,⁴¹⁶ Deanesly and Parkes,⁴³⁸ Koch,⁴¹⁶ Ruzicka,⁴³⁶ and Tschopp.⁴¹⁹ The natural hormones are printed in boldface type.

† Where the symbol Δ is used the ending "-ane" should be changed to "-ene." The C_3 -OH is referred to the C_{10} -CH₃. The spatial configuration of the C_{17} -OH groups is open to question.

‡ International comb units.

§ Rat units.

|| Has estrogenic activity.

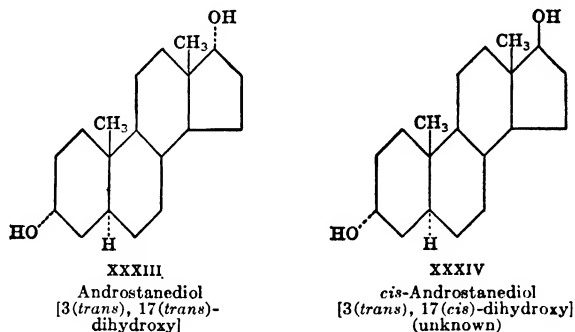
¶ Needles.

** Leaflets.

1–2.5 mg. per liter,⁴⁴¹ of which about 6 per cent can be isolated in pure form; the yield of dehydroandrosterone from urine is about 200 mg. from 1000 liters of urine.⁴⁴²

Androsterone is usually prepared by warm chromic acid oxidation of the acetate of *epidi*hydrocholesterol, but the conversion, cholesterol \rightarrow dihydrocholesterol \rightarrow cholestanone \rightarrow *epidi*hydrocholesterol, using the acetates, has certain technical difficulties due to the limited solubility of some of the products.* Marker⁴⁴³ has suggested a more convenient method in which cholesteryl chloride is the starting material; after hydrogenation and oxidation, chlorine is replaced with hydroxyl by treatment with potassium acetate to give directly an *epi*-C₃—OH group. (Cf. Walden inversion, p. 1259.) Androsterone has also been prepared from *epicinchol* by chromic acid oxidation⁴⁴⁴ and from *allolithocholic acid* by Barbier-Wieland degradation.⁴⁴⁵

Relatively few compounds with male hormone activity have been prepared from androsterone. By reduction, either catalytically⁴⁴⁶



or with sodium and propyl alcohol,⁴⁴⁷ androstanediol [3(*trans*), 17-(*trans*?)-dihydroxy] (XXXIII) is formed and by reaction with methyl-

⁴⁴¹ Callow, *Lancet*, **231**, 565 (1936).

⁴⁴² See Dannenbaum, reference 416. Publications by Kochakian (*Endocrinology*, **21**, 60 (1937)), Dingemans, Borchardt, and Laqueur [*Biochem. J.*, **31**, 500 (1937)], and Peterson, Gallagher, and Koch [*J. Biol. Chem.*, **119**, 185 (1937)] indicate that these values are too low, or do not account for all the androgenic material in normal urine.

* The overall yield of androsterone from cholesterol may reach 0.2 per cent under favorable conditions. For a detailed study of the preparation see Callow and Deanesly, *Biochem. J.*, **29**, 1424 (1935).

⁴⁴³ Marker, *J. Am. Chem. Soc.*, **57**, 1755 (1935); Marker, Whitmore, and Kamm, *ibid.*, **57**, 2358 (1935).

⁴⁴⁴ Dirscherl, *Z. physiol. Chem.*, **237**, 52, 268 (1935).

⁴⁴⁵ Dalmer, Werder, Honigmann, and Heyns, *Ber.*, **68**, 1814 (1935).

⁴⁴⁶ Butenandt and Tscherning, *Z. physiol. Chem.*, **234**, 224 (1934).

⁴⁴⁷ Ruzicka, Goldberg, and Meyer, *Helv. Chim. Acta*, **18**, 994 (1935); Ruzicka and Goldberg, *ibid.*, **19**, 99 (1936).

or ethylmagnesium iodide the 17-methyl-⁴⁴⁷ or 17-ethyl-androstanediol results;⁴⁴⁸ with the Grignard reagents there is also some reduction (pp. 427, 556) of the 17-keto group to carbinol.* Both androstanediol and 17-methylandrostanediol are about three times more potent physiologically than androsterone. Cold chromic acid oxidation of androsterone converts it to androstanedione (XXVIII), but there is little change in potency through the conversion of the C_3-OH to a ketonic group. On treatment with bromine,† androstanedione is substituted at C_2 , and on heating the monobromo product with potassium acetate in acetic acid at 200° , hydrogen bromide is removed to give Δ^1 -androstenedione.⁴⁴⁹ In contrast to the other androstenediones described below, Δ^1 -androstenedione is without male hormone activity but produces estrus in a daily dose of 500 γ over a period of four days. Androstanedione reacts with two molecules of alkyl Grignard reagents, but the resulting 3,17-dialkylandrostanediols are not potent.⁴³⁸

Androgenic Hormones from Dehydroandrosterone. Chromic acid oxidation of dibromocholesteryl acetate at $50-60^\circ$, followed by debromination, gives a 2.8 per cent yield of dehydroandrosterone.⁴²⁴ This is the best way of preparing the compound, although it has been prepared from stigmasterol⁴²⁴ and sitosterol.⁴⁵⁰ If the intermediary product of this oxidation, 5,6-dibromoisoandrosterone, is oxidized to a diketone prior to debromination, the bromine can be removed to give either an α,β - or a β,γ -unsaturated ketone. Removal of bromine from the dibromo hydroxy ketone is accompanied by a rearrangement to Δ^4 -androstenedione when zinc and acetic acid are employed,⁴⁵¹ but when zinc and weakly acid alcohol are used rearrangement does not occur and Δ^5 -androstenedione is formed.⁴⁵² There is a marked difference in the physiological potency of these isomers, as is seen from the data of Table XIII; unfortunately precise data are not available for the comb test with the Δ^5 -enedione. The difference in physiological potency due to a shift of the double bond from $C_4 : C_5$ to $C_5 : C_6$ is brought out

⁴⁴⁸ Ruzicka and Rosenberg, *ibid.*, **19**, 357 (1936).

* In this and similar cases reduction does not occur with methyl Grignard reagents, takes place to a small extent with the ethyl Grignard reagent, and is the predominant reaction with the isopropyl reagent.

† On bromination the ketones derived from androstane are substituted to give products similar to those obtained with the sterol ketones (p. 1273). When androstanedione is treated with bromine a dibromo compound can be obtained and on heating, HBr and methane are split out to give what is presumably isoequinin, m.p. 252° , $[\alpha]_D + 170$. Physiologically isoequinin has an activity comparable to that of equilin. [Inhoffen, *Naturwissenschaften*, **25**, 125 (1937).]

⁴⁴⁹ Butenandt and Dannenberg, *Ber.*, **69**, 1158 (1936).

⁴⁵⁰ Oppenauer, *Nature*, **135**, 1039 (1935).

⁴⁵¹ Butenandt and Kudzus, *Z. physiol. Chem.*, **237**, 75 (1935).

⁴⁵² Butenandt and Schmidt-Thomé, *Ber.*, **69**, 882 (1936).

more clearly in the pairs, testosterone acetate and Δ^5 -testosterone acetate,⁴⁵³ or Δ^4 -androstenediol and Δ^5 -androstenediol.^{430, 431} The ethylenic double bond of the β,γ -unsaturated ketones is rearranged very easily to the α,β -position; this is shown by the fact that Δ^5 -testosterone acetate gives testosterone on hydrolysis.⁴⁵³

When Δ^5 -androstenedione is reduced catalytically in the presence of the Raney-nickel catalyst, a mixture of dehydroandrosterone and *epi*-dehydroandrosterone is obtained.⁴⁵⁴ The two compounds are easily separated by precipitating dehydroandrosterone with digitonin. This method is the only known way of preparing *epidehydroandrosterone*, since it cannot be prepared from *epicholesterol* in a manner analogous to that used for dehydroandrosterone from cholesterol; on attempting to form the dibromide, a tetrabromo compound is obtained.⁴⁵⁵

With Grignard reagents the esters of dehydroandrosterone react to give the 17-alkyl derivatives and at the same time a side reaction involving the reduction of the C_{17} carbonyl to carbinol takes place.⁴⁵⁶ The by-product is a mixture of diols from which two compounds may be separated by fractional crystallization. In the higher-melting more soluble diol the C_{17} -OH group probably has a *cis* configuration with respect to the C_{13} -CH₃ group, and the compound is appropriately called *cis*- Δ^5 -androstenediol. The lower-melting diol agrees in properties with Δ^5 -androstenediol, formed by reduction of dehydroandrosterone by sodium and alcohol, or catalytically.⁴⁵⁷

The principal products from the Grignard reactions, the 17-alkyl-androstenediols, serve as sources of a number of important compounds. In the case of the 17-methyl-androstenediol conversion has been made to 17-methylisoandrostanediol,⁴³⁶ 17-methyltestosterone,⁴³⁶ and 17-methyldihydrotestosterone.⁴³⁶ The latter two compounds are especially important because of their high potencies both in the capon test and in the rat test. In fact, 17-methyldihydrotestosterone is regarded by Deanesly and Parkes⁴³⁵ as the most potent of the androgens, either natural or synthetic, when all the effects are considered.

Androgenic Hormones from Isoandrosterone. Isoandrosterone, being more easily prepared than androsterone, is generally used for the preparation of androstenedione. On catalytic reduction in acid media, or on reduction with sodium and propanol, isoandrosterone

⁴⁵³ Butenandt and Hanisch, *Ber.*, **69**, 2773 (1936).

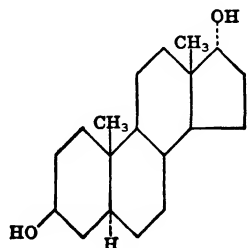
⁴⁵⁴ Ruzicka and Goldberg, *Helv. Chim. Acta*, **19**, 1407 (1936).

⁴⁵⁵ Marker, Kamm, Oakwood, and Laucius, *J. Am. Chem. Soc.*, **58**, 1948 (1936).

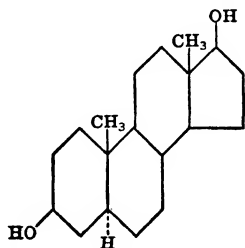
⁴⁵⁶ Ruzicka and Rosenberg, *Helv. Chim. Acta*, **19**, 357 (1936); Butenandt, Cobler, and Schmidt, *Ber.*, **69**, 448 (1936).

⁴⁵⁷ Ruzicka, Goldberg, and Meyer, *Helv. Chim. Acta*, **18**, 210 (1935); Ruzicka and Wettstein, *ibid.*, **18**, 1270 (1935).

is converted to isoandrostanediol (XXXV).^{436, 458} Ruzicka formulates this compound as one in which the C₁₇—OH group is *trans* to the C₁₃—CH₃, and according to his practice of referring the C₃—OH group to the C₅—H, the compound should be described as 3(*trans*),17(*trans*)-dihydroxyandrostane. According to the convention of referring the C₃—OH group to the C₁₀—CH₃, isoandrostanediol is described as



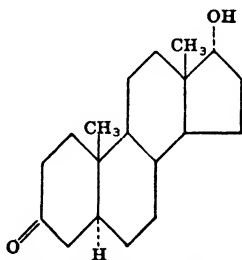
XXXV
Isoandrostanediol
[3(*cis*), 17(*trans*)-dihydroxy]



XXXVI
cis-Isoandrostanediol
[3(*cis*), 17(*cis*)-dihydroxy]

3(*cis*),17(*trans*)-dihydroxyandrostane. The isomeric *cis*-isoandrostanediol (XXXVI), with the C₁₇—OH group in a *cis* relationship to the C₁₃—CH₃, has been prepared by the catalytic reduction of *cis*- Δ^5 -androstenediol.⁴⁴⁰

Dihydrotestosterone (XXXVII) is readily available from isoandrosterone or testosterone.⁴⁵⁹ The preparation from isoandrosterone is carried out *via* the diacetate of isoandrostanediol. Saponification at room temperature with alcoholic alkali takes place preferentially to



XXXVII Dihydrotestosterone

give 3-hydroxy-17-acetoxyandrostane, and this compound is easily converted to dihydrotestosterone by oxidation and saponification. Testosterone when hydrogenated in absolute ether with palladium sponge as a catalyst is converted directly to dihydrotestosterone. A

⁴⁵⁸ Butenandt, Tscherning, and Hanisch, *Ber.*, **68**, 2097 (1935); *cf.*, Ruzicka and Goldberg, *Helv. Chim. Acta*, **19**, 99 (1936).

⁴⁵⁹ Butenandt, Tscherning, and Hanisch, *Ber.*, **68**, 2097 (1935).

trans configuration at C₅ is produced in this reduction which is in accord with v. Auwers-Skita's rule but contrary to the experience of obtaining coprostanone from cholestenone (p. 1257).

Structure and Physiological Action. It was appreciated early in the study of the androgenic hormones that there are two types of hormones of which the typical representatives are androsterone and testosterone. The androsterone type is characterized by a lower value for the ratio, comb unit : rat unit, than the testosterone type. Possibly the ideal male hormone would be one in which this ratio is 1 : 1. Of the known androgens, isoandrostanediol most nearly approaches this ideal, but, unfortunately, this hormone suffers from the handicap that it possesses low activity both in the capon and in the rat tests.

Various points in connection with structure and physiological activity have been developed in the course of the discussion of the methods of preparation of the male hormones. They may be recapitulated and extended here with the aid of the generalizations that have been made by Deanesly and Parkes.⁴³⁵

1. A normal configuration of the C₃—OH group (*trans* to C₅—H, *cis* to C₁₀—CH₃) is unfavorable to comb growth and may be unfavorable to seminal vesicle growth; an *epi* configuration of the C₃—OH group is favorable to comb growth. Examples: Isoandrosterone (C.U., 700–850) *vs.* androsterone (C.U., 100), or isoandrostanediol (C.U., 520–600) *vs.* androstanediol (C.U., 23–33).

2. Oxidation of an *epi*-C₃—OH group to ketone has little effect on the activity, but oxidation of the normal C₃—OH to ketone increases the activity markedly. Examples: Androsterone (C.U., 100), androstanedione (C.U., 100–120), and isoandrosterone (C.U., 700–850); or androstanediol (C.U., 23–33), dihydrotestosterone (C.U., 25–30), and isoandrostanediol (C.U., 520–600).

3. Reduction of the 17-keto group to hydroxyl increases the male activity, especially that on the seminal vesicles. A *trans* configuration of the C₁₇—OH group is more potent than a *cis* configuration. Examples: Δ^4 -Androstenedione (R.U., 500 *) and testosterone (R.U., 100); androstanedione (R.U., 700) and dihydrotestosterone (R.U., 50); testosterone (R.U., 16) and *cis*-testosterone (R.U., >1000).

4. Unsaturation at C₄ or C₅ may increase the seminal vesicle activity, but the effect is irregular: Example with increase: Isoandrosterone (R.U., 5000) and dehydroandrosterone (R.U., 3000). Example with decrease: Dihydrotestosterone (R.U., 50), and testosterone (R.U., 100).

* The values for the rat unit (R. U.) are from Butenandt (Table XIII) except for the pair testosterone, *cis*-testosterone of item 3, where the values of Tschopp are used.

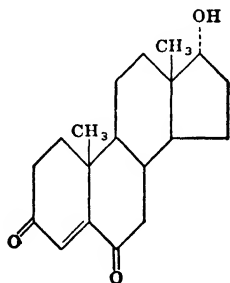
5. A methyl group at C₁₇ may increase the comb activity and definitely increases the activity on the prostate gland. Example: Methylisoandrostanediol (C.U., *ca.* 400), and androstanediol (C.U., 23-33).

6. Alkylation at C₃ (C₃—R) destroys the activity completely.

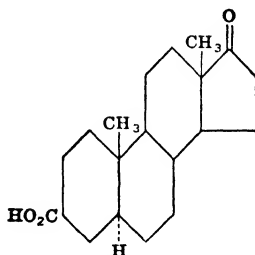
Activators and Inactivators. When testosterone is combined with the inactive oils obtained from urine, the resulting activity is greater than that of testosterone alone.⁴²⁹ The study of this phenomenon has led to the discovery of substances that activate, or inactivate, the male hormones. The saturated fatty acids such as palmitic acid serve as activators,⁴⁶⁰ but paraffin hydrocarbons and glycerol inactivate to the extent of about 50 per cent.⁴⁶⁰ Propylene glycol, on the other hand, increases the activity of testosterone.⁴⁶¹

The esters of testosterone have been investigated also.⁴⁶² In general the esters are favorable to seminal vesicle growth rather than to comb growth. The maximum activity was obtained by the use of the esters of the butyric acids and *n*-valeric acid, although the propionate is definitely more potent. With these esters the ratio of the size of the prostate to the seminal vesicles is the same as in normal animals; with testosterone the ratio is in favor of the prostate. Like the esters of the estrogenic hormones the duration of the physiological action is somewhat prolonged with the esters of the male hormones.

Bisexual Hormones. As cited above, Δ^1 -androstenedione is active estrogenically but is devoid of male hormone activity. Two other



XXXVIII 6-Oxotestosterone
(steric position of
C₁₇—OH provisional)



XXXIX *cis*- and *trans*-
3-Carboxyandrostanone

compounds have been found that are structurally related to the androgens but which have estrogenic activity comparable to that of the true estrogens. These are 6-oxotestosterone (XXXVIII), formed by the

⁴⁶⁰ Miescher, Wettstein, and Tschopp, *Schweiz. Med. Wochschr.*, **66**, 310 (1936).

⁴⁶¹ Deanesly and Parkes, *Lancet*, **231**, 837 (1936).

⁴⁶² Miescher, Wettstein and Tschopp, *Biochem. J.*, **30**, 1977 (1936).

oxidation of the C₁₇-acetate of Δ^4 -androstenediol,^{462a} and the mixture of *cis* and *trans* forms of 3-carboxyandrostanone (XXXIX), obtained when the Grignard from the chloride of dehydroandrosterone is treated with carbon dioxide.⁴⁵⁵ The three androgenic hormones, Δ^5 -androstenediol, dehydroandrosterone, and Δ^5 -17-methylandrostenediol, not only have male hormone activity but also produce estrus.⁴⁶² The enol esters of the androgens with unsaturation at C₄ are without estrogenic activity even though ring A is semi-benzenoid in structure in these compounds.⁴⁶³ Nearly all the androgens that are non-estrogenic in the Allen-Doisy test do produce vaginal opening (proestrus) in immature animals and may be said to have estrogenic tendencies.* Surprising as this estrogenic activity is, the production of progestational proliferation by 17-methyltestosterone and testosterone⁴⁶⁴ is even more remarkable, since in a sense this biological phenomenon is more truly female than estrus.† It is uncertain in all these responses whether the action is direct or indirect.

The Biogenesis of the Sex Hormones

With the realization of the close relationship that exists structurally between cholesterol and the sex hormones, both Ruzicka and Butenandt have formulated transformations showing how the hormones can conceivably originate from the sterol. The scheme that has been developed by Ruzicka³⁶⁴ is shown in the accompanying diagram. In this scheme the names of the native hormones are shown in boldface type; the naturally occurring compounds that are related to the hormones, in italics; and a few related intermediary compounds not found in nature, in ordinary type. The degradations from one stage to another are supposedly accomplished by enzymatic oxidation, reduction, and

^{462a} Butenandt and Riegel, *Ber.* **69**, 1163 (1936).

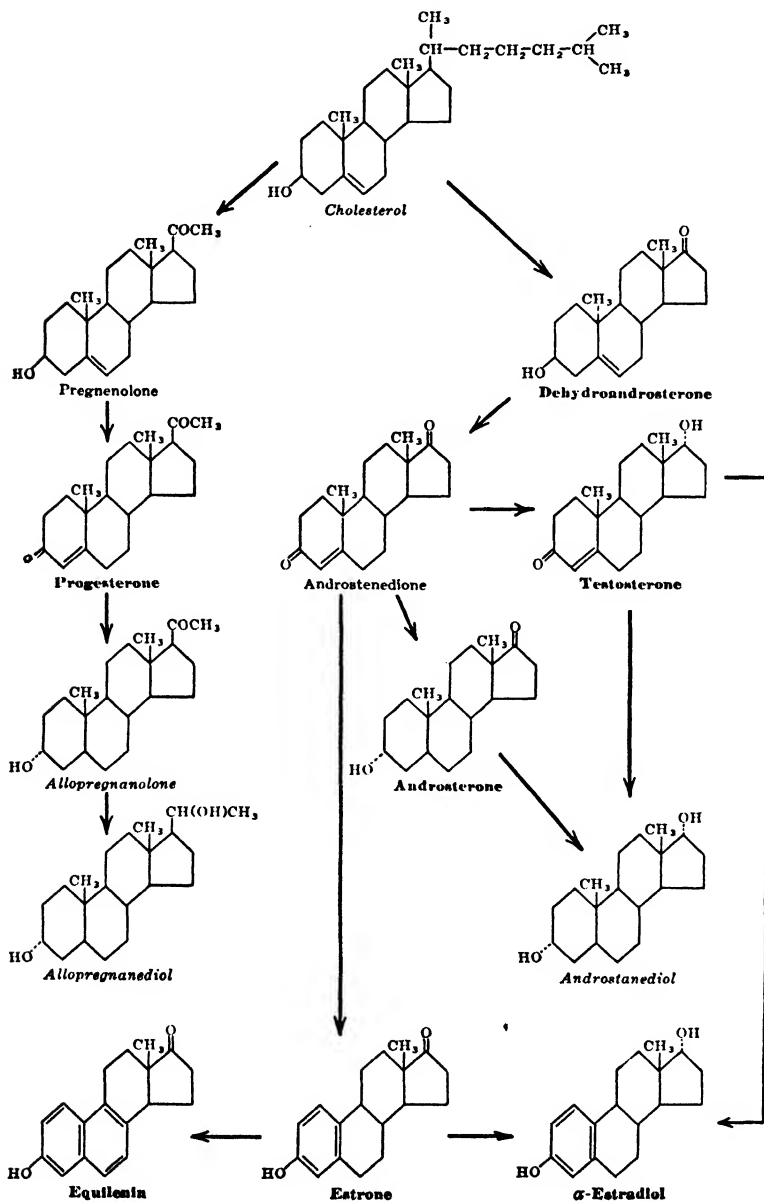
⁴⁶³ Ruzicka and Fischer, *Helv. Chim. Acta*, **19**, 1371 (1936).

* For list of compounds with positive vaginal test, see Dannenbaum, *Ergeb. Physiol.*, **38**, 832-833 (1936).

⁴⁶⁴ Klein and Parkes, *J. Soc. Chem. Ind.*, **55**, 236 (1936); *Proc. Roy. Soc. (London)*, **121B**, 574 (1937).

† It has been pointed out by Korenchevsky and co-workers, *Biochem. J.*, **31**, 780 (1937) (and earlier papers), that, aside from progesterone, there are no purely "male" or "female" hormones. Furthermore, there are relatively few "true" bisexual hormones, and according to this investigator only dehydroandrosterone, testosterone, and Δ^4 -androstenediol really qualify for this group. The other hormones may be regarded as partially bisexual. Androsterone, testosterone propionate, and androstenediol are bisexual hormones with chiefly "male" characteristics. Estrone and estradiol are bisexual hormones with chiefly "female" properties. In reaching these conclusions, Korenchevsky has studied the effects of the hormones on the entire genital system. The various hormones have been tested singly and in combination.

demethylation. The schemes are plausible, but they do not take into account the mechanism of the formation of cholesterol in the body. It is possible that a modification of the same cellular mechanism that



produces cholesterol may be responsible for the production of the sex hormones.*

THE ADRENAL SUBSTANCES†

Two important hormones, adrenaline and cortin, are produced in the adrenals, small glands, one situated near each kidney. The production of cortin is the more important function of the adrenals, since it is essential to life and is apparently produced only in these glands. Adrenaline, although also essential to life, is produced in several other organs. A deficit of cortin caused by injury to the adrenals leads to bronzing of the skin, muscular weakness, and an increase in the blood urea level; overdevelopment of the glands in children results in precocious sexual development. The last fact suggests that cortin is either closely related to the sex hormones or is involved in their production.‡

* In support of a biochemical transformation of cholesterol to sex hormones the work of Rondoni, Carminati, and Corbellini, *Z. physiol. Chem.*, **241**, 71 (1936), may be cited. These workers allowed mixtures of cholesterol and minced liver to autolyze at various temperatures for several weeks. At the end of this time extracts of the mixtures were made and found to have estrogenic activity in some instances, but apparently the tests were not well controlled [Cf. Voss and Rabald, *ibid.*, **245**, 76 (1937) and Rondoni, *ibid.*, **245**, 78 (1937)]. In a later publication, Rondoni and co-workers, *ibid.*, **247**, 225 (1937) reaffirm their results.

The conversion of one sex hormone to another by yeast has been studied by Mamoli. Top yeast converts dehydroandrosterone to Δ^5 -androstenediol [Mamoli and Vercellone, *ibid.*, **245**, 93 (1937)] and under reducing conditions yeast hydrogenates Δ^4 -androstenedione to testosterone [Mamoli and Vercellone, *Ber.*, **70**, 470 (1937)]. Although in these cases the point of attack is the grouping at C₁₇, the action is not limited to this center since androstenedione is reduced to isoandrostenediol by yeast [Vercellone and Mamoli, *Z. physiol. Chem.*, **248**, 277 (1937)].

† For summary see Reichstein, *Helv. Chim. Acta*, **19**, 29 (1936) and Mason, Hoehn, McKenzie, and Kendall, *J. Biol. Chem.*, **120**, 719 (1937). For physiology see Grollman, "The Adrenals," Williams and Wilkins, Baltimore (1936).

‡ The interrelation of the sex hormones and the adrenal substances is further indicated by the isolation of a number of unusual compounds from the urine of patients with pathological changes of the adrenals. The first case reported was one of a girl with an adrenal tumor who showed signs of virilism. In this instance, Callow, *J. Soc. Chem. Ind.*, **55**, 1030 (1936), found that abnormally high amounts of dehydroandrosterone were excreted. Later, Butler and Marrian, *J. Biol. Chem.*, **119**, 565 (1937), studied the urine of two young women suffering from the adreno-genital syndrome and were able to isolate a new compound, pregnan-3,17,20-triol, C₂₇H₄₆O₃, m. p. 243–244°. This triol does not form an insoluble digitonide and on gentle oxidation with lead tetraacetate yields acetaldehyde and 3-*epi*-hydroxyetiocolan-17-one. Thus the C₃—OH is *trans* to the C₁₀—OH₁, and the compound may be described as 3 (*trans*),17,20-trihydroxypregnane. Perhaps it bears the same relationship to one of the adrenal substances as pregnanediol does to progesterin.

On the other hand, Burrows, Cook, Roe, and Warren, *Biochem. J.*, **31**, 950 (1937), have isolated $\Delta^{3,5}$ -androstadien-17-one from the urine of a man with a tumor of the adrenal cortex. This patient showed definite signs of femininity and excreted large amounts of estrogenic substances but not enough for characterization. $\Delta^{3,5}$ -Androstadien-17-one may be regarded as having some biological connection with dehydroandrosterone from

Methods of obtaining crude potent extracts were developed in 1929-1930.* Since then several groups of investigators have attempted to isolate the hormone, but without success. In the course of the search for the pure hormone a number of substances have been encountered that are probably closely allied with it. All the substances obtained in sufficient quantity for examination have been found to be closely related to the androgenic hormones, and several of them to possess male hormone activity.

The cortin content of extracts and of purified fractions may be evaluated by testing on animals whose adrenals have been removed. Either adrenalectomized dogs or rats are used for the assay. For dogs, one cortin unit is the minimum daily dose per kilogram which will maintain the blood urea level and the weight in essentially normal condition when administered over a period of seven days. With rats a fatigue test is generally used. The rat unit is equivalent to 50-100 dog units. Although most of the testing is done on rats, the dog method is far more exact. The purest cortin preparations which have been reported assay about 400 dog units per milligram.

The Adrenal Substances. In the extraction of cortin the adrenals are treated with alcohol or acetone, the solution freed of adrenaline, and the fats removed by partition between hydrocarbon solvents and water, or water-alcohol solutions. Active and inactive compounds are finally separated by fractional crystallization, by partition between solvents,† or by the application of Girard's Reagent T (p. 1357). By these methods Mason, Myers, and Kendall⁴⁶⁵, ⁴⁷⁵ have obtained eight compounds, designated by the letters A-H; Pfiffner and Wintersteiner,⁴⁶⁶ seven compounds, A-G; and Reichstein,⁴⁶⁷, ⁴⁷⁴ twelve compounds, A-M. The chemical composition and physical constants of the several compounds are listed in Table XIV. Through exchange of material and by comparison of physical constants a number of identities have been estab-

which it is easily prepared by dehydration with anhydrous copper sulfate at 200°. This same patient excreted *p*-cresol in his urine. Since publication of this fact, Marshall, *Nature*, **140**, 362 (1937), has reported that pregnant mares excrete a considerable amount of this phenol, 110 grams of *p*-cresol having been isolated from 400 gallons of gravid mares' urine.

* The best method appears to be that of Swingle and Pfiffner, *Medicine*, **11**, 371 (1932). Other methods have been developed by Hartman and Brownell, Grollman, and Rogoff and Stewart; for summaries see "Ann Rev. Biochem.," Vols. I-V.

†According to Kendall, reference 475, the compounds containing 4 oxygen atoms may be separated from those containing 5 oxygen atoms by distribution between benzene and water, the O₄ compounds remaining in the benzene.

⁴⁶⁵ Mason, Myers and Kendall, *J. Biol. Chem.*, **114**, 613 (1936); **116**, 267 (1936).

⁴⁶⁶ Pfiffner, Wintersteiner, and Vars, *J. Biol. Chem.*, **111**, 585 (1935); **116**, 291 (1936).

⁴⁶⁷ Reichstein, *Helv. Chim. Acta*, **19**, 29, 1107 (1936).

TABLE XIV
 ADRENAL SUBSTANCES

Compound	Formula	M. P., ° C.	$[\alpha]_{446}$ (alcohol)
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Series of Mason, Myers, and Kendall

A	$C_{21}H_{20}O_4$	177-180	$+299 \pm 1$
B	$C_{21}H_{30}O_4$	177-180	$+258 \pm 3$ *
C	$C_{21}H_{34}O_5$	245-250	$+ 84 \pm 5$
D	$C_{20}H_{34-36}O_5$	214-216	$+ 29$ (acetone)
E	$C_{21}H_{30}O_5$	201-208	$+248 \pm 4$
F	$C_{21}H_{30}O_5$	214-220	$+178 \pm 5$
G	$C_{21}H_{32}O_5$	228-236	$+ 83 \pm 2$
H	$C_{21}H_{32}O_4$	172-176	$+118$

Compound	Formula	M. P., ° C.	$[\alpha]_D$ (alcohol)
----------	---------	-------------	------------------------

Series of Pfiffner and Wintersteiner

A	$C_{20-21}H_{34-36}O_5$	214	
B	$C_{21}H_{32}O_5$	210-214	
C	$C_{24}H_{40}O_7$	125-128	
D	236-239	
E	$C_{11}H_{18}C_2N_2$	158-161	
F	$C_{29}H_{28}O_5$	203-209	$+209$
G	$C_{21}H_{34}O_3$	264	$+ 38$

Compound	Formula	M. P., ° C.	$[\alpha]_D$ (absolute alcohol)
----------	---------	-------------	---------------------------------

Series of Reichstein

A	$C_{21}H_{34-38}O_5$	221-222 c.	$+16$
B	253-255 c.	
C	$C_{21}H_{32-36}O_5$	253-256 c.	$+ 69.8 \pm 2.5$
D	$C_{21}H_{32-36}O_5$	230-238 c., d.	$+ 66 \pm 1.5$
E	$C_{21}H_{30-32}O_5$	126-127 c.	$+ 87 \pm 2$
Fa	$C_{21}H_{28-30}O_5$	215 c.	$+200 \pm 8$
G	$C_{17-19}H_{22-26}O_3$	216-223 c.	$+262$
H	$C_{21}H_{26-32}O_{4-5}$	180-182 c.	$+223$
I	$C_{21}H_{34-36}O_3$	216-217 c.	
K		175 c.	
L		255 c.	
M		205-208 c.	

* $[\alpha]_D(alc.) = + 222$.

lished. In the tabulation given below the compounds listed in the same vertical column are identical.

Kendall's Series.	B	C	E	G	
Pfiffner & Wintersteiner's Series.			F	D	A
Reichstein's Series.	H	C	Fa		A

All the compounds are ketones or hydroxyketones. In the impure state cortin activity is destroyed by alkali and, since crude extracts of progesterone and testosterone are also inactivated by this treatment, it appears from the outset that there is a similarity of structure between the adrenal hormone and the sex hormones. Partial formulas for many of these compounds can be given, but in all cases one oxygen atom remains unassigned. There is evidence that this oxygen atom is attached at C₁₁.

Reichstein's Substance A. The first compound to be degraded to a recognizable product was compound A of Reichstein's series.⁴⁶⁸ On oxidation with periodic acid an hydroxyketone (II), m.p. 236°, is formed. By further oxidation with chromic acid the diketone (III), m.p. 178°, is obtained, and, by reduction of this diketone with amalgamated zinc and catalytic hydrogenation, a mixture of androstane (IV) and 17-hydroxyandrostane (V) is produced. Androstanedione, reduced by the same procedure, also gives these two end products. The diketone III is formed from substances C and D of Reichstein's series, if they are oxidized with chromic acid. From the investigations of Clutterbuck⁴⁶⁹ as well as of other workers⁴⁷⁰ the reactions with periodic acid and with lead tetraacetate indicate that the structure at C₁₇ is essentially that of glycerol. Thus Reichstein's compound A has structure I with one unassigned oxygen which may be present in an unreactive carbonyl group, in an inert hydroxyl group, or in an oxide ring.

Reichstein's Substances E and G. The fractions with greatest cortin activity absorb in the ultra-violet at 240 mμ (α,β -unsaturated ketones), and from these fractions a number of ketones with absorption in the same region have been isolated. Reichstein's substances E and G are two such compounds. Substance G is active in the capon comb test and has been given the name adrenosterone and the probable structure VII.⁴⁷¹ Substance E is converted to substance G by chromic acid oxidation; it probably has the configuration shown in structure VI.⁴⁷²

⁴⁶⁸ Reichstein, *ibid.*, **19**, 402, 979 (1936).

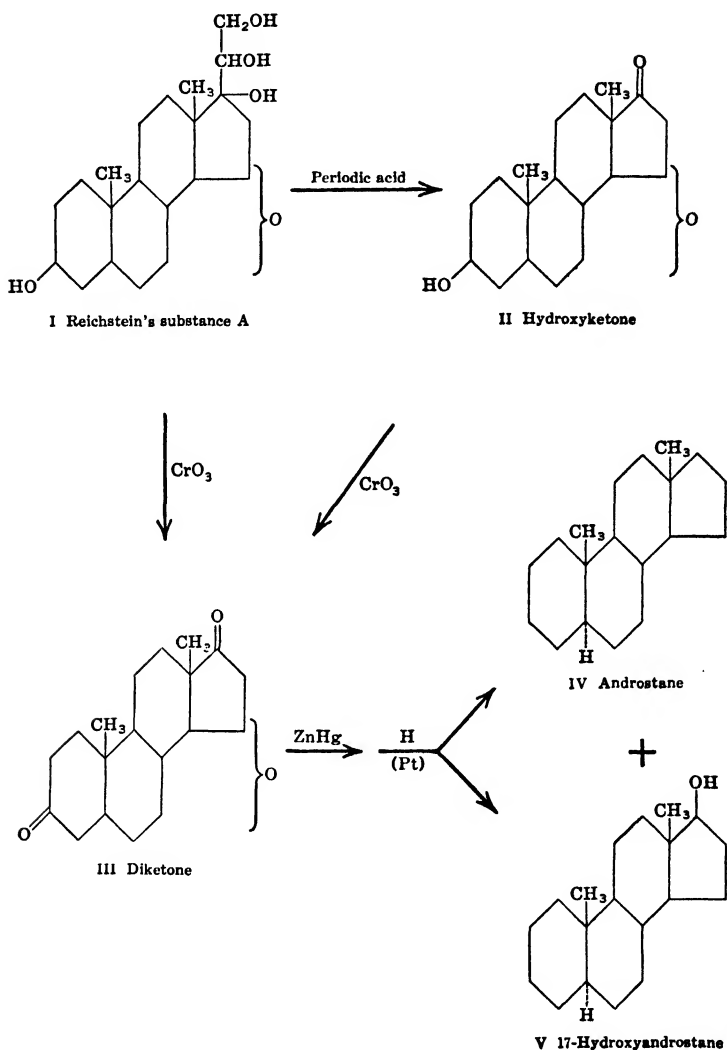
⁴⁶⁹ Clutterbuck and Reuter, *J. Chem. Soc.*, 1467 (1935).

⁴⁷⁰ e.g. Criegee, *Ann.*, **495**, 211 (1932).

⁴⁷¹ Reichstein, *Helv. Chim. Acta*, **19**, 223 (1936).

⁴⁷² Reichstein, *ibid.*, **19**, 1107 (1936).

Reichstein's Substance Fa—Kendall's Substance E. Although Reichstein's substance Fa and Kendall's substance E appear to be

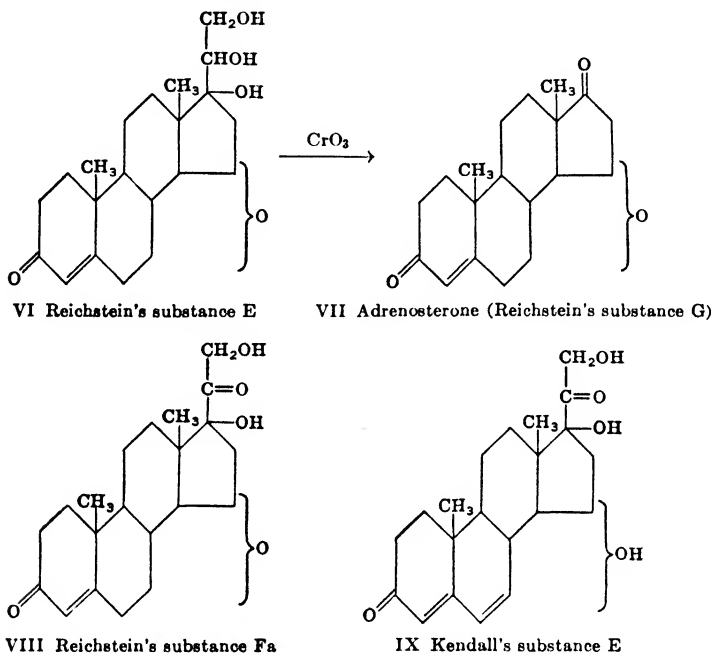


identical, two different structures have been offered for the compound. Reichstein⁴⁷² has suggested structure VIII on the basis of absorption spectrum and the products of oxidative degradation; Kendall⁴⁷³ interprets similar evidence to indicate structure IX. For his compound

⁴⁷² Mason, Myers, and Kendall, *J. Biol. Chem.*, **116**, 267 (1936).

Kendall claims qualitative cortin activity, but the other investigators do not agree with this conclusion.

Kendall's Substance B—Reichstein's Substance H: Corticosterone. A compound with cortin activity approximately one-tenth that of the purest cortin extract has been described both by Reichstein⁴⁷⁴ and by



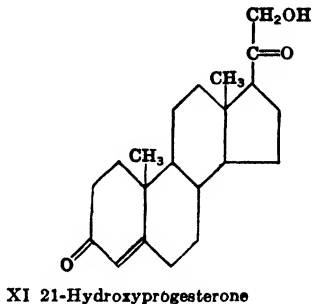
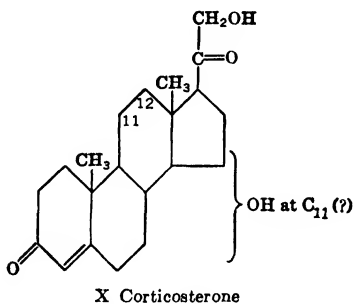
Kendall⁴⁷⁵ and has been named corticosterone by the former. Formula X gives the partial structure of corticosterone. The presence of a

$\text{C}_{17}-\overset{\text{O}}{\parallel}\text{C}-\text{CH}_2\text{OH}$ side chain has been established by oxidizing corticosterone with periodic acid. The products are an acid ($\text{C}_{19}\text{H}_{27}\text{O}_2$)— CO_2H (Kendall's acid 2, XII) and formaldehyde. Corticosterone gives the reactions of a diketone and absorbs in the ultraviolet at $240\text{ m}\mu$. The second carbonyl group has been assigned to C_3 in conjugation with a double bond at C_4 . The most convincing argument for the correctness of this formulation comes from the work of Reichstein.⁴⁷⁴

⁴⁷⁴ DeFremery, Laqueur, Reichstein, Spanhoff, and Uylert, *Nature*, **139**, 26 (1937); Reichstein, *ibid.*, **139**, 331 (1937); Steiger and Reichstein, *ibid.*, **139**, 925 (1937).

⁴⁷⁵ Mason, Hoehn, McKenzie, and Kendall, *Abstracts Organic Section, 93rd Meeting Am. Chem. Soc.*, p. 17; Kendall, Mason, Hoehn, and McKenzie, *Proc. Staff Meetings Mayo Clinic*, **13**, 136, 270 (1937); Mason, Hoehn, McKenzie, and Kendall, *J. Biol. Chem.*, **120**, 719 (1937).

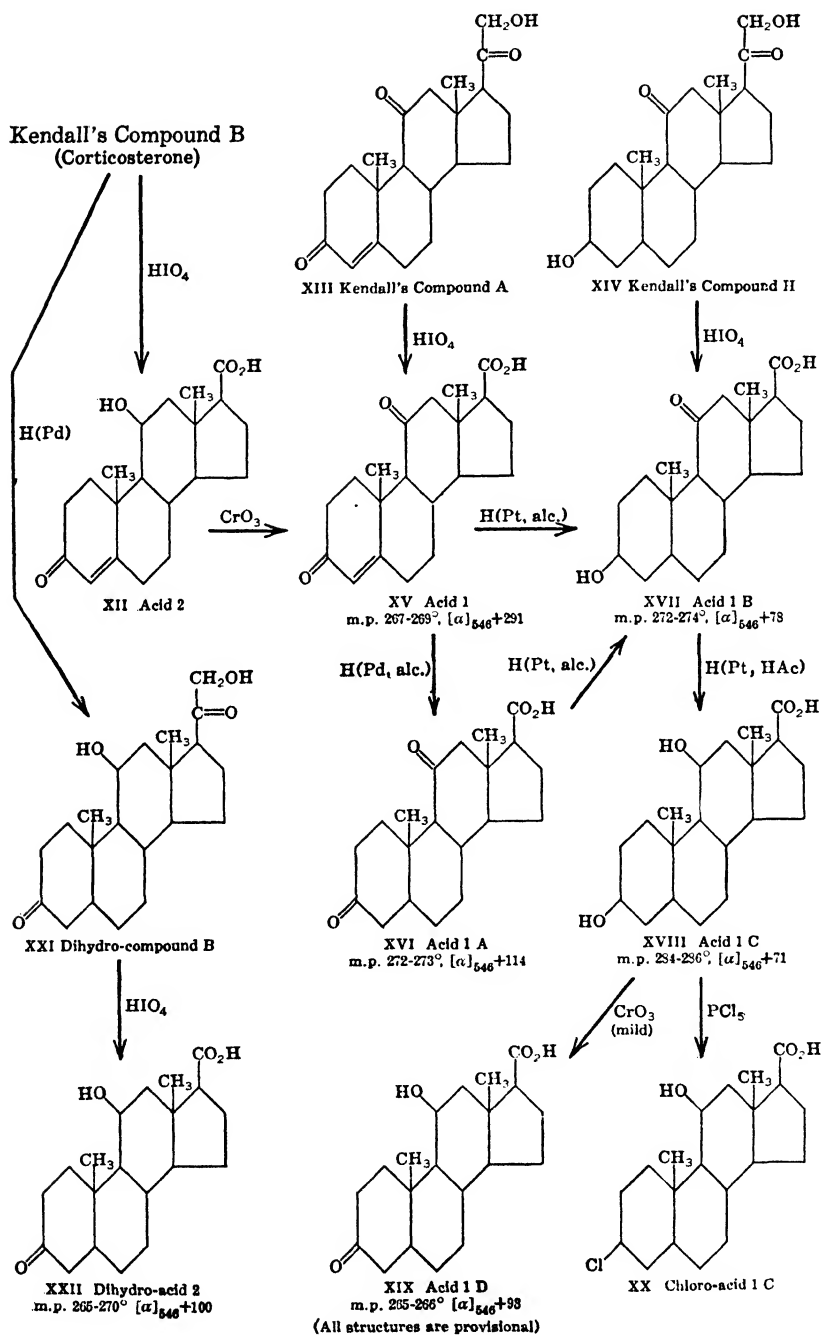
This investigator degraded stigmasterol (p. 1283) to 21-hydroxyprogesterone (XI), a compound which is analogous in structure to that proposed for corticosterone. When administered to an adrenalectomized dog, 21-hydroxyprogesterone was capable of maintaining the animal in good condition, but the required level of dosage was three times that of corticosterone. Other evidence indicating the presence of a carbonyl group at C₃ in corticosterone is discussed below.



The Unassigned Oxygen. The position of the unassigned oxygen is difficult to establish because of the extremely small amounts of the natural compounds that are available for study,* and because of the unreactive character of the functions containing this oxygen. The accumulated evidence indicates that the unplaced oxygen is present at C₁₁. This conclusion has been reached from the study^{474, 475} of the two physiologically active compounds, Kendall's A (XIII) and corticosterone, and of the inactive compound, Kendall's compound H (XIV).

When compound A (XIII) of Kendall's series is oxidized with periodic acid, formaldehyde and acid 1 (XV) are formed. Acid 1 is also obtained when acid 2 (XII) is oxidized with chromic acid. Acid 1 reacts with methylmagnesium iodide (Kohler method, p. 416) in such a way that one carboxyl and two carbonyl groups are indicated, and the absorption spectrum of acid 1 shows that one of these carbonyl groups is conjugated with an ethylenic double bond. When acid 1 is hydrogenated in slightly alkaline alcohol with palladium as a catalyst, acid 1A (dihydro-acid 1, XVI) is produced. Hydrogenation of acid 1A, or of acid 1, in alcoholic solution with platinum as a catalyst results in the uptake of another mole of hydrogen to give acid 1B (XVII) which may be further hydrogenated in acetic acid solution to acid 1C (hexahydro-acid 1, XVIII). Acids 1B and 1C give insoluble digitonides in 90 per cent alcohol. With the Grignard reagent, acid 1C shows 2.5 active

* For example, 0.7–1.0 gram of compound A was obtained from 3000 pounds of fresh glands (Kendall, reference 475).



hydrogens and probably has two hydroxyl groups—one at C₃, the other at C₁₁. The hydroxyl group that is assigned provisionally to C₁₁ is inert, however, for on mild oxidation with chromic acid in aqueous acetone it is not attacked, and, on treatment with PCl₅ in organic solvents, it is not substituted by chlorine. In both reactions the C₃—OH is acted upon to give, in the first instance, the ketonic acid 1D (XIX); in the second, the chloro-acid 1C (XX).

The inactive compound, Kendall's substance H, differs from compounds A and B in that it does not absorb ultra-violet light and forms an insoluble digitonide. On oxidation with periodic acid, compound H is converted to acid 1B, the product of catalytic hydrogenation with platinum of acid 1. Since this and the other hydrogenations discussed above are carried out in neutral media, and the products form insoluble digitonides, the several compounds probably correspond structurally to dihydrocholesterol rather than to coprosterol (*cf.*, p. 1257).

The inertness of the unplaced oxygen function is suggestive of the behavior of the other compounds of the steroids—digoxigenin and sarmantogenin and their derivatives (pp. 1333, 1335)—with oxygen at C₁₁ and cannot be reconciled with attachment at any other position, except possibly at C₁₂. To rule out the possibility of attachment at C₁₂, Kendall prepared 3,12-diketotiocholanic acid, m.p. 156–159°, for comparison with acid 1 (XV). The two acids are obviously different but, as Kendall later recognized, they are probably isomeric about C₅ and the comparison is meaningless. Reichstein,⁴⁷⁶ also, sought to eliminate attachment at C₁₂ by studying the properties of a compound prepared from corticosterone, in which only the unplaced oxygen function was present, presumably as an hydroxyl group. Because of the uncertainty of whether or not an hydroxyl group was actually present in the substance that was studied, the argument is not wholly convincing and will not be considered here.

A further puzzling situation arises in connection with acid 1D. If corticosterone is catalytically reduced in the presence of palladium, one mole of hydrogen is taken up and dihydro-compound B (XXI) results. On oxidation with periodic acid, the α -ketol side chain of dihydro-compound B is converted to carboxyl, but the product, dihydro-acid 2 (XXII), is not identical with acid 1D even though the physical constants are very close. The lack of identity is shown by the fact that a mixed melting point of the methyl esters of the two acids is *ca.* 25° below that of the methyl ester of either pure acid, both of which melt at 170–171°. Evidently the two acids are stereoisomeric about C₁₁.

⁴⁷⁶ Steiger and Reichstein, *Helv. Chim. Acta*, **20**, 817 (1937).

Obviously generalizations on the relation of structure and physiological activity cannot be made at the present time. It is apparent, however, that a carbonyl group at C₃ in conjugation with a double bond at C₄ is essential for high activity. Kendall⁴⁷⁵ reports that reduction of the C₄ ethylenic linkage reduces the physiological activity to 1/50 of that of the parent compounds.

Other Adrenal Substances. Wintersteiner and Pfiffner⁴⁷⁷ have provisionally identified their substance E as leucylproline anhydride. Reichstein has isolated a sulfur-containing substance, C₄H₁₀O₃S (not listed in the series). It has been identified as bis-(β -hydroxyethyl)-sulfoxide.⁴⁷⁸ Although these substances have no bearing on the cortin problem, their occurrence, especially that of the sulfoxide, is interesting.

CONCLUSION

The determination of the structures of the various members of the cyclopentanoperhydrophenanthrene group is a brilliant example of modern methods of investigation. It should be emphasized that the essential clue to the final rapid development of the field was furnished by the timely suggestion of Rosenheim and King, based on the x-ray measurements (p. 1229) of Bernal. Thus the impetus to clarification in one of the most important chapters of organic chemistry came from physical measurements.

Obviously the structural chemistry of this group of compounds is not concluded. Although the arguments leading to the accepted structures are extremely plausible, synthetic evidence is still lacking, and until synthetic proof is available, some uncertainty must remain. Already a great deal of work is in progress toward this goal, but as yet it is in a preliminary stage. The fascinating problem of how these compounds are formed in nature is equally important, and with this is associated the question of how minor modifications in the structure result in such different physiological effects. Indeed, viewed as a whole, the chemistry of the cyclopentanoperhydrophenanthrene group begins, rather than ends, with the determination of acceptable structural details.

GENERAL REFERENCES

The reviews and monographs dealing with the individual members of the cyclopentanoperhydrophenanthrene group have been given as the first reference in each section. The entire field has been covered in the following books: Fieser, "The Chemistry of Natural Products Related to Phenanthrene" (A.C.S. monograph 70),

⁴⁷⁷ Wintersteiner and Pfiffner, *J. Biol. Chem.*, **111**, 599 (1935).

⁴⁷⁸ Reichstein and Goldschmidt, *Helv. Chim. Acta*, **19**, 401 (1936).

Reinhold Publishing Corp., New York (1937), 456 pages, 2nd ed.; and Lettré and Inhoffen, "Über Sterine, Gallensäuren und verwandte Naturstoffe," Enke, Stuttgart (1936), 320 pages. Although the context is similar in the two books, Fieser portrays the difficulties encountered and the nature of the investigation in greater detail than Lettré and Inhoffen. The two German authors have incorporated a large number of experimental details in their book; the section on ergosterol and its transformation products is especially well documented.

A separate monograph on the chemistry and physiology of the bile acids has appeared: Shimizu, "Über die Chemie und Physiologie der Gallensäuren," Muramoto, Okayama (1935), 388 pages. In this book some 82 pages are devoted to the chemistry of the bile acids.

From time to time the physical constants of some of the families of compounds have appeared in the publication *Tabulae Biologicae Periodicae*. References to these tables will be found in the text.

In addition to these newer publications, various reviews have appeared in Abderhalden's "Biochemisches Handlexikon" and "Handbuch der biologische Arbeitsmethoden" and in Oppenheimer's "Handbuch der Biochemie." Most of these discussions are written from the older point of view and are of value largely for experimental details and physical constants. The later volumes (Ergänzungsbande) of Oppenheimer deal with current phases, however.

The more important reviews are listed below:

Structure

WINDAUS, *Z. physiol. Chem.*, **213**, 147 (1932).

HEILBRON, SIMPSON, and SPRING, *J. Chem. Soc.*, 626 (1933).

Sterols

BILLS, *Physiol. Rev.*, **15**, 1 (1935).

HEILBRON, *J. Soc. Chem. Ind.*, **55**, 129T (1936).

Bile Acids

SOBOTKA, *Chem. Rev.*, **15**, 311 (1934).

Cardiac Aglucons and Toad Poisons

ELDERFIELD, *Chem. Rev.*, **17**, 187 (1935).

TSCHESCHE, *Ergeb. Physiol.*, **38**, 31 (1936).

STOLL, "The Cardiac Glycosides," The Pharmaceutical Press, London (1937).

Digitalis Sapogenins

TSCHESCHE, *Ergeb. Physiol.*, **38**, 65 (1936).

Estrogenic Hormones

STÖRMER and WESTPHAL, *Ergeb. Physiol.*, **35**, 318 (1933).

Corpus Luteum Hormone

ALLEN, *Science*, **82**, 89 (1935).

WESTPHAL, *Ergeb. Physiol.*, **37**, 273 (1935).

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DANNENBAUM, *Ergeb. Physiol.*, **38**, 796 (1936).

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KOCH, *Physiol. Rev.*, **17**, 153 (1937).

CHAPTER 16

CARBOHYDRATES I

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INTRODUCTION

The research field concerned with the development of the fundamental organic chemistry of the carbohydrates has been a very active one and is still the object of a large amount of research. The great organic chemist Emil Fischer made his first mark here, and many others have been attracted by this fascinating group of substances. The carbohydrates may be classified from the standpoint of their hydrolytic products into monosaccharides, oligosaccharides, and polysaccharides; the last two groups produce monosaccharides on hydrolysis; the molecular complexity of the oligosaccharides is known with certainty, but that of the polysaccharides is still an uncertain quantity. The monosaccharides are polyhydroxy aldehydes and ketones that reduce mild alkaline reducing agents, such as Fehling's solution. They may be further classified according to the length of their carbon chain and according to the nature of their carbonyl function; thus there are aldopentoses, aldohexoses, ketohexoses, etc. The monosaccharides are colorless crystalline solids and possess a sweet taste.

The central compound of the carbohydrates is *d*-glucose, and any development of the subject of carbohydrate chemistry from a research problem standpoint must revolve about this substance. *d*-Glucose is a monosaccharide classified as an aldohexose. It is the most readily available of the monosaccharides and the most important one from the standpoint of animal metabolism. Since all the monosaccharides are polyhydroxy aldehydes or ketones, it follows that knowledge gained by an investigation of the *d*-glucose molecule can generally be extended to its many relatives. This extension is not always easily accomplished, and of course significant and interesting differences in reactivity are exhibited by the various other monosaccharides. The development of our present conception of the structure of *d*-glucose represents a fascinating chapter in the evolution of a chemical formula. As new experimental evidence was obtained, previous ideas had to be revised

in the sense that the old views were not wrong but were incomplete. Thus the formula of *d*-glucose stands today as a representation of one of the most thoroughly investigated substances in the entire field of organic chemistry.

The original sweetening agent native to Europe was the sugar mixture known as honey. Alexander the Great is credited with introducing cane sugar into Europe from the Orient, and purified cane sugar or sucrose was undoubtedly the first crystalline sugar known. Ironically, of all the sugars, the formula of this substance has proved to be the most difficult to unravel. Since the sugar cane could be grown only in the tropics, a search was made in Europe for a native plant substitute amenable to field cultivation, and this culminated successfully in the beet-sugar industry, established by Achard¹ (1798). In the course of this search, Marggraf² (1747) had crystallized a substance which he recognized as being different from cane sugar and which he termed "eine Art Zucker." This was the substance which is now called glucose. Sucrose crystallizes very readily. Glucose, on the contrary, is a difficult substance to crystallize, and it is only within the past few years that crystalline glucose has been produced commercially at a low cost.

The writings of Marggraf do not constitute the first record of crystalline glucose. This had been prepared previously from a variety of sources, but especially from grapes, and was known to the ancient Persians and Arabians. Reference to this grape sugar can be found in the old Moorish records³ (1150) and in the writings of the alchemists and early pharmacists.

Elementary analysis of glucose produced the empirical formula CH_2O . This formula represents the origin of the French term *hydrate de carbone*, which was modified in the German to *Kohlenhydrat* and the latter translated into English as carbohydrate. A molecular-weight determination showed that the true formula was $\text{C}_6\text{H}_{12}\text{O}_6$. This result was not obtained until 1888 (Tollens and Mayer),⁴ as no method was available for determining its molecular weight until the appearance of the work of Raoult⁵ (1880) and the Beckmann apparatus⁶ (1888).

That glucose contained five acetylable hydroxyl groups was proved

¹ Achard, "Die europäische Zuckerfabrikation aus Runkelrüben," Hinrichs, Leipzig (1809); cf., von Lippmann, "Geschichte des Zuckers," 2nd ed., Springer, Berlin (1929), pp. 698, 700, 701.

² Marggraf, *Ber. Berliner Akad.*, V, 79 (1749); cf., von Lippmann, "Geschichte des Zuckers," 2nd ed., Springer, Berlin (1929), p. 683.

³ Ibn Al-Awam, II, 398, 400; cf., von Lippmann, "Geschichte des Zuckers," 2nd ed., Springer, Berlin (1929), p. 682.

⁴ Tollens and Mayer, *Ber.*, 21, 1566 (1888).

⁵ Raoult, *Ann. chim. phys.*, [5] 20, 217 (1880).

⁶ Beckmann, *Z. physik. Chem.*, 2, 638 (1888).

definitely by Franchimont⁷ (1879 and 1892), who obtained its first crystalline pentaacetate. This, together with its reducing properties, gave the formula $C_5H_7(OH)_5-CO$. It is necessary to emphasize here that real progress in the chemistry of the sugars can result only on the basis of pure crystalline derivatives. The failure to recognize this premise adequately has led to many grievous errors and much confusion.

Kiliani⁸ then improved the procedure of Schützenberger⁹ (1881) for forming the cyanohydrin of glucose, hydrolyzed this to the acid, reduced the latter with hydriodic acid, and obtained *n*-heptoic acid (1886). This proved that an aldehyde group was present and that the six carbon atoms of glucose were arranged in a straight or normal chain, thus indicating the formula $CH_2OH-(CHOH)_4-CHO$. Kiliani¹⁰ (1886) also applied the above procedure to fructose, which was an isomer of glucose isolated by Dubrunfaut¹¹ (1856) from invert sugar through its property of forming an insoluble compound with lime. Kiliani arrived at the formula $CH_2OH-(CHOH)_3-CO-CH_2OH$ for this substance and thus demonstrated the existence of a polyhydroxy ketone or ketose.

CONFIGURATIONAL ISOMERISM OF THE MONOSACCHARIDES

It is to be noted that the Kiliani formula for glucose contained four asymmetric carbon atoms and according to the van't Hoff¹²-Le Bel¹³ (1874) theory, which was at that time quite new, there were 2^4 or sixteen possible isomers for this compound. Examples of such isomers had begun to appear, as for instance, the isolation of galactose in 1856 by Louis Pasteur.¹⁴ The simple sugars that were isolated from natural sources and found to be different from glucose were considered to be isomeric with it and assigned the same formula. One of these supposed isomers was arabinose, and on closer investigation of this substance Kiliani¹⁵ (1886) was surprised to find that it contained only five carbon atoms. Thus was established the first member of the group of sugars known as the aldopentoses. The problem of determining the spatial configuration of all these isomers appeared quite hopeless. To add to

⁷ Franchimont, *Ber.*, **12**, 1940 (1879); *Rec. trav. chim.*, **11**, 106 (1892).

⁸ Kiliani, *Ber.*, **19**, 767 (1886).

⁹ Schützenberger, *Bull. soc. chim.*, [2] **36**, 144 (1881).

¹⁰ Kiliani, *Ber.*, **19**, 221 (1886).

¹¹ Dubrunfaut, *Compt. rend.*, **42**, 901 (1856).

¹² van't Hoff, "Sur les formules de structure dans l'espace," *Arch. néerland. sci.* (1874).

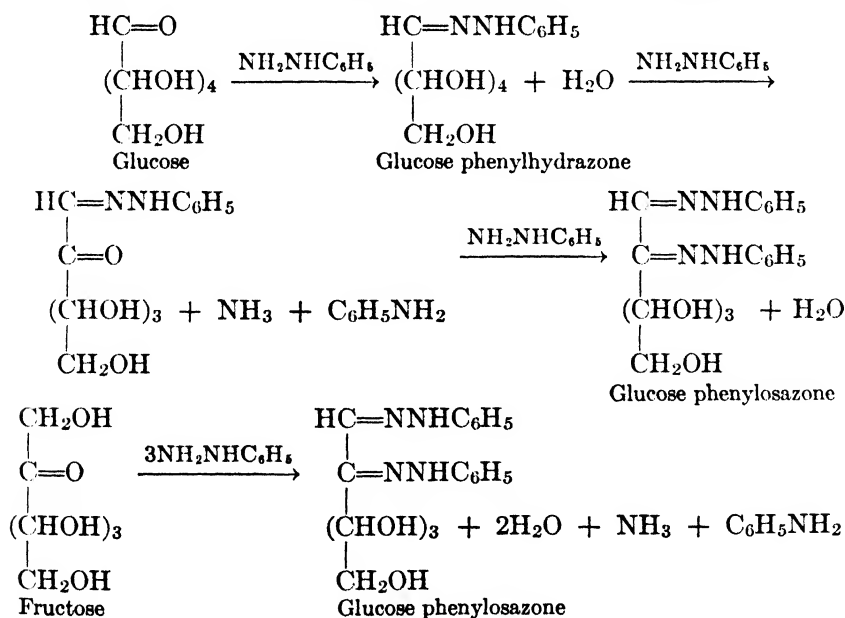
¹³ Le Bel, *Bull. soc. chim.*, [2] **22**, 367 (1874).

¹⁴ Pasteur, *Compt. rend.*, **42**, 347 (1856).

¹⁵ Kiliani, *Ber.*, **19**, 3029 (1886); **20**, 339 (1887).

the difficulty, the substances and their derivatives were difficult to crystallize, and they were also very sensitive to heat and to strong reagents. The time was thus ripe for a genius to arise who would possess the ability and industry to bring order out of chaos, and this genius was Emil Fischer.

Emil Fischer was at the time concerned with the apparently unrelated problem of preparing substitution products of hydrazine, among which was phenylhydrazine. He noted that the latter compound served quite well for preparing derivatives of aldehydes and ketones and he tried its action with the sugars¹⁶ (1884). Certainly he must have been astonished to find that crystalline substances were formed with surprising ease. However, these did not analyze correctly for phenylhydrazones, two phenylhydrazine residues having entered the molecule. On further investigation¹⁷ (1887) he found that oxidation on the carbon atom adjacent to the aldehyde group had taken place with the formation of aniline, ammonia, and the substance he termed the phenylosazone. He was able to find the true phenylhydrazone as the expected intermediate, this substance being in most cases too soluble for ready separation. He noted¹⁸ (1884) that the phenylosazone obtained from glucose was identical with that obtained from fructose, and a start was made on the problem of solving the spatial configuration of the sugars.

¹⁶ Fischer, *Ber.*, **17**, 579 (1884).¹⁷ Fischer, *Ber.*, **20**, 821 (1887).¹⁸ Fischer, *Ber.*, **17**, 579 (1884).

Sugars producing the same osazone thus had identical structures on all but the first two carbon atoms. The aldohexose *d*-mannose also yielded *d*-glucose phenylosazone and accordingly differed from glucose only in the configuration of the carbon atom adjacent to the aldehyde group. Aldoses bearing such a relationship are now termed epimers (p. 181), a name suggested by Votoček¹⁹ (1911).

The three fundamental procedures used by Fischer (1884–1894) in his great feat of elucidating the configuration of the sugars were osazone formation, oxidation to *meso* acids or reduction to *meso* alcohols, and the methods for building up or degrading the members of the sugar series. The formation of the *meso* or optically inactive and unresolvable compounds indicated the symmetrical or internally compensated structures (p. 168). The reactions involved in the Fischer procedures will be discussed in some detail before their application to the solution of configurational problems is illustrated. These procedures constitute fundamental reactions of the aldoses and have been used for the synthesis of the various members of the aldose series.

These aldose synthetic methods employ a naturally occurring sugar as the starting point and transform this into other aldoses by various procedures. Fischer did attain a complete glucose synthesis by developing the experiments of Butlerow²⁰ (1861), who had noted that formaldehyde and alkali produce sugars. This interesting reaction was further investigated by Fischer. He obtained a low yield of racemic glucose phenylosazone from the mixture²¹ (1889) and then skillfully completed the difficult steps from racemic glucose phenylosazone to *d*-glucose²² (1890). Since formaldehyde can be synthesized from its elements, a complete glucose synthesis was thus accomplished.

The aldose oxidation techniques were initiated mainly by Kiliani. On hypobromite oxidation, the aldoses produce the corresponding aldonic acids, a reaction that was greatly simplified by Isbell²³ (1930), who produced the hypobromite ion by continuous electrolysis in a sugar solution containing a small amount of bromide ion, hydrobromic acid being the reduction product and this in turn being electrolyzed to form again hypobromite ion. Nitric acid oxidizes an aldose to a dibasic hydroxy acid, the aldehyde group and the terminal primary alcohol being the points of attack.

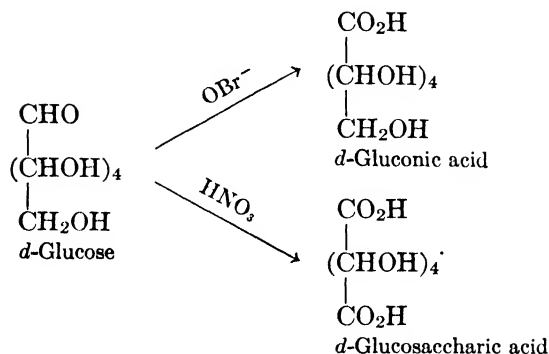
¹⁹ Votoček, *Ber.*, **44**, 362 (1911).

²⁰ Butlerow, *Ann.*, **120**, 295 (1861).

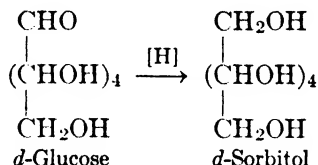
²¹ Fischer and Passmore, *Ber.*, **22**, 359 (1889).

²² Fischer, *Ber.*, **23**, 370, 799 (1890).

²³ Isbell and Frush, *Bur. Standards J. Research*, **6**, 1145 (1930).



The aldoses may also be reduced to the corresponding sugar alcohols by various reducing agents, such as sodium amalgam. The reaction proceeds very slowly with chemical reducing agents, and at present the most promising procedures are those involving electrolytic methods or high-pressure catalysis²⁴ (Ipatieff, 1912).



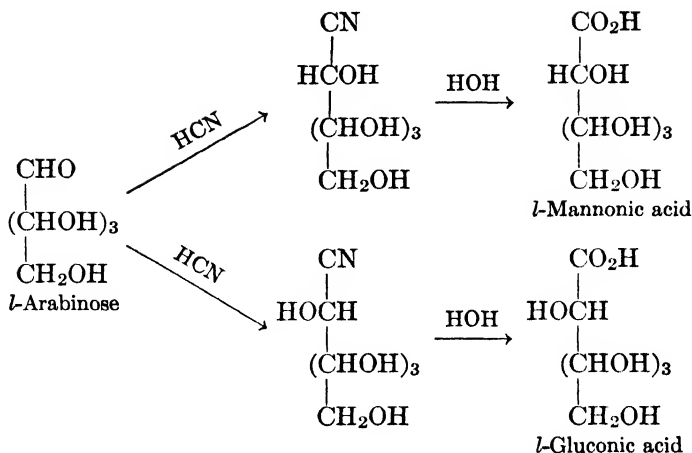
The cyanohydrin procedure for adding a carbon atom to an aldose had been developed by Kiliani, as previously noted. He had always isolated only one product from the reaction. For example, the cyanohydrin reaction when applied by him to arabinose²⁵ (1886 and 1887) produced the lactone of a new hexonic acid, later found to be *l*-mannonic. On repetition of this work Fischer²⁶ (1890) found the two products required by theory, one of which was the enantiomorph of *d*-gluconic acid. The two products can be predicted because a new asymmetric carbon atom is formed. They will be formed in unequal amounts as a new asymmetric center has been added to a molecule already asymmetric (p. 166).

The aldonic acids undergo lactonization with ease, and this is the form in which they are generally obtained although a number of the

²⁴ Ipatieff, *Bull. soc. chim.*, [4], **14**, 552 (1913); *Ber.*, **45**, 3218 (1912).

²⁵ Kiliani, *Ber.*, **19**, 3029 (1886); **20**, 339 (1887).

²⁶ Fischer, *Ber.*, **23**, 2611 (1890).



free acids have been prepared by crystallization from solvents in which lactone formation is hindered (Rehorst,²⁷ 1928). Kiliani²⁸ (1887) made the remarkable observation that the double lactone of mannosaccharic acid could be reduced to the sugar alcohol mannitol with sodium amalgam. Fischer²⁹ (1889) found that this reaction was general for the sugar lactones and made an important extension by finding that, when the procedure was carried out under slightly acidic conditions, the reduction stopped at the aldose stage. In this way Fischer obtained the epimeric higher carbon sugars of *d*-glucose, calling the one that crystallized α -glucoheptose and the one that did not β -glucoheptose. By repeating the process by which glucose was changed to a heptose, Fischer³⁰ (1892) was able to make a glucononose, and Philippe³¹ (1912) carried this to the decose stage.

In addition to the above-described synthesis of epimers through the cyanohydrin reaction and reduction of the resultant lactones, Fischer also employed the aldonic acids directly for epimer synthesis. This important general reaction resulted from his discovery³² (1890) that an aldonic acid is converted in part into its epimeric acid on heating its solution with a mild base, such as pyridine. The two acids could then be separated and the desired epimeric lactone reduced to the aldose.

²⁷ Rehorst, *Ber.*, **61**, 163 (1928); **63**, 2279 (1930).

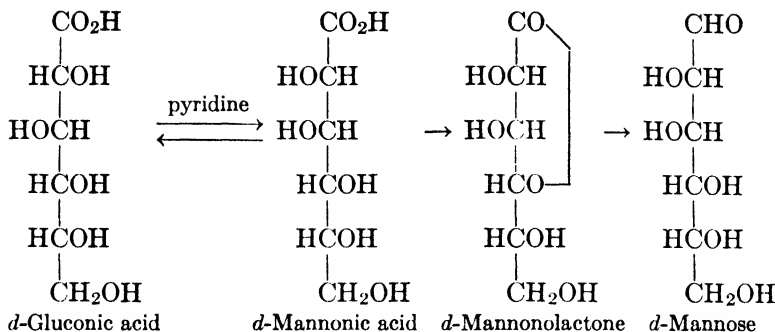
²⁸ Kiliani, *Ber.*, **20**, 2714 (1887).

²⁹ Fischer, *Ber.*, **22**, 2204 (1889).

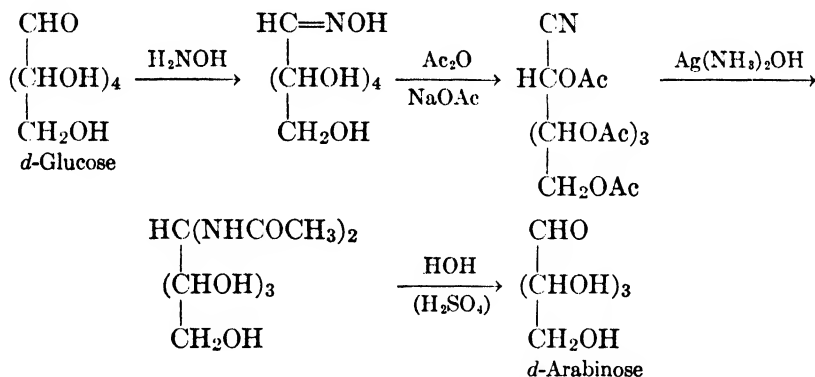
³⁰ Fischer, *Ann.*, **270**, 64 (1892).

³¹ Philippe, *Ann. chim. phys.*, [8] **26**, 289 (1912).

³² Fischer, *Ber.*, **23**, 799 (1890).



The sugar degradation methods extended and confirmed the facts obtained by the cyanohydrin reactions. The first method used was that developed by Wohl. Wohl³³ (1893) acetylated *d*-glucose oxime and obtained gluconic acid nitrile pentaacetate. Treatment of this substance with an ammoniacal solution of silver oxide produced the diacetamide compound of arabinose, and this on acid hydrolysis yielded *d*-arabinose or the enantiomorph of the common naturally occurring pentose.



This procedure gives fair results with most of the monosaccharides, and Zemplén later extended it to the disaccharides by replacing the ammoniacal silver solution with sodium ethylate. In this manner Zemplén³⁴ (1926) degraded the disaccharides cellobiose and lactose by one carbon atom. The next degradation method was developed by Ruff³⁵ (1898) and is the one that has been the more successful for preparative purposes. It is a modification of the Fenton³⁶ (1893)

³³ Wohl, *Ber.*, **26**, 730 (1893).

³⁴ Zemplén, *Ber.*, **59**, 1254, 2402 (1926).

³⁵ Ruff, *Ber.*, **31**, 1573 (1898).

³⁶ Fenton, *Proc. Chem. Soc.*, **9**, 113 (1893).

reaction. The calcium salt of the sugar acid is treated with hydrogen peroxide in the presence of ferric ion, and degradation to the next lower aldose is effected. Weerman³⁷ (1918) developed a degradation method based upon the action of hypochlorite upon the amide of the sugar acid, but this extension of the old Hofmann reaction (p. 759) has not received much application.

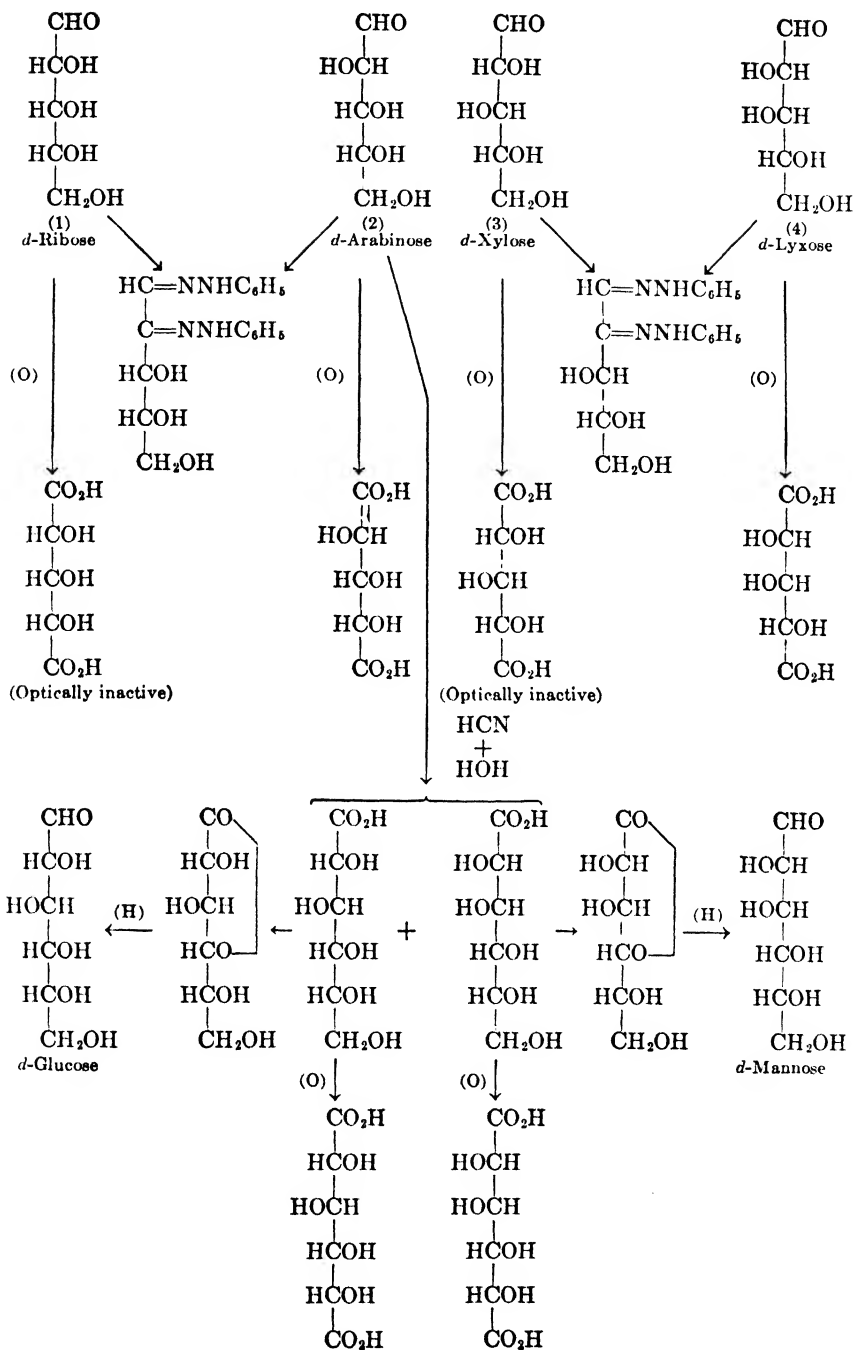
Having described the important procedures available to Emil Fischer, their use will be illustrated by indicating how they were applied in determining the structure of the five carbon aldoses or pentoses. Eight (2³) active forms are theoretically possible, and the *d*- and *l*-forms of arabinose, xylose, lyxose, and ribose were the compounds eventually made known through the labors of the Fischer school and others. The possible configurations are the four given (p. 1409) and their enantiomorphs.

Since arabinose and ribose give the same osazone and are consequently epimeric, they must be either (1) and (2) or (3) and (4). Lyxose and xylose likewise give identical osazones. Arabinose on nitric acid oxidation gives an optically active trihydroxyglutaric acid, hence arabinose can have only the configuration (2) or (4). Furthermore, arabinose with hydrogen cyanide and subsequent hydrolysis and oxidation gives two *active* dicarboxylic acids, and must therefore have the structure (2), as one of the acids derived in this way from (4) would be optically inactive by internal compensation. If (2) represents *d*-arabinose, then its enantiomorph represents *l*-arabinose, and (1) must represent *d*-ribose. Xylose yields an inactive trihydroxyglutaric acid when oxidized and must then be represented by configuration (3), and hence lyxose is (4). Since *l*-arabinose, when treated with hydrogen cyanide and the product hydrolyzed and reduced, yields a mixture of *l*-glucose and *l*-mannose, it follows that in these last two the spatial arrangement of their carbon atoms three to five, inclusive, is identical with that of *l*-arabinose. This is confirmed by the fact that *d*-glucose produces *d*-arabinose on degradation by one carbon atom and thus *d*-arabinose is configurationally related to dextrorotatory or *d*-glucose.

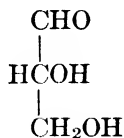
In the above reasoning no indication is given regarding which of the enantiomorphs of each pentose corresponds to the assigned numbers. Fischer used *d*-glucose and the tartaric acids as his reference compounds, and this led to ambiguities in the gulose-idose aldohexose series which arose from Fischer's naming the gulose obtained from *d*-glucose as the *d*-form. Rosanoff³⁸ (1906) has shown that in order to obtain a solution of this problem which is free from ambiguities it is

³⁷ Weerman, *Rec. trav. chim.*, **37**, 16 (1918).

³⁸ Rosanoff, *J. Am. Chem. Soc.*, **28**, 114 (1906).



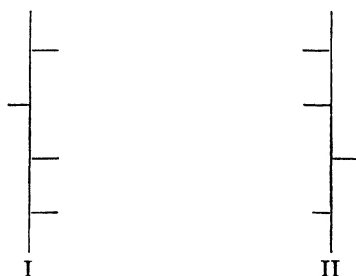
necessary to choose the aldose containing only one asymmetric center as the ultimate reference substance. This substance is glyceraldehyde, whose *d*-form is represented empirically below.



It is to be emphasized that the symbols *d*- and *l*- refer to configuration and not to sign of rotation, the conventions (dextro) and (levo) denoting the latter (p. 220). The symbol *d,l*- will be used to indicate a racemic form and *i*- to denote the inactive and non-resolvable *meso* form. It is well known that *d*(dextro)-glucose is configurationally related to (levo)-fructose, both giving the same phenylosazone. Accordingly the common form of fructose is *d*(levo)-fructose.

Rosanoff considered all the higher *d*-aldoses as being derived from *d*-glyceraldehyde by successive cyanohydrin syntheses. Accordingly an aldose belongs to the *d*-series when the hydroxyl group on the carbon directly attached to the end primary alcohol group is represented on the right in the stereochemical projection formula. The elaboration of the aldose series according to Rosanoff is given in Fig. 1. The conventional representation is that used by Rosanoff in which a horizontal line to the right indicates a hydroxyl group in that direction and the top circle represents the aldehyde group. These conventional representations may be rotated only in the plane of the paper.

According to the Rosanoff classification, the same dibasic or saccharic acid is obtained from *d*-glucose and *l*-gulose.



The representation of the saccharic acid from *d*-glucose is indicated by (I) and that from *l*-gulose by (II). The latter when rotated 180° in the plane of the paper is identical with (I). The configuration symbol thus loses its significance in this one case, and the long-known compound

prepared by Scheele³⁹ (1776) is then best represented merely as (dextro)-saccharic acid.

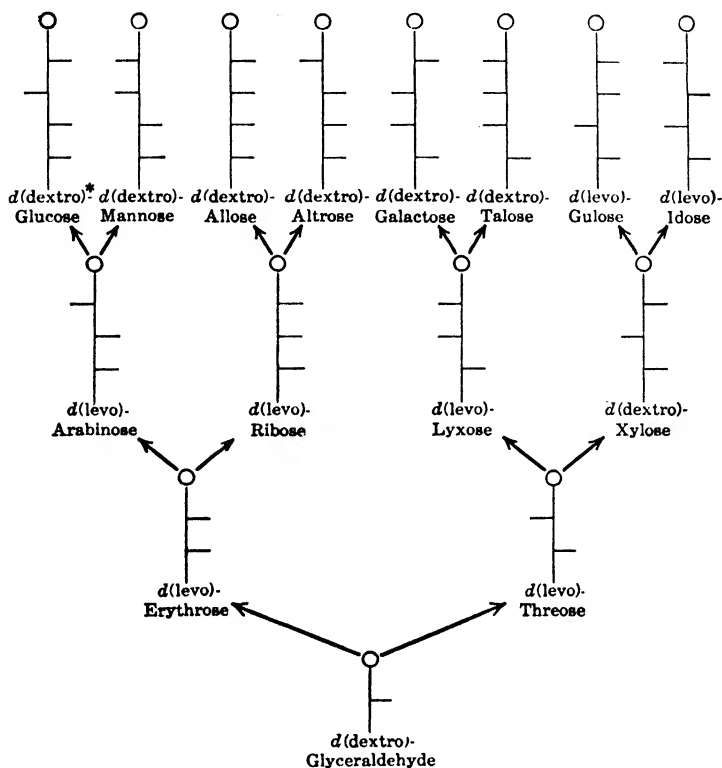


FIG. 1.—*d*—Series of the aldoses

Glyceraldehyde is a synthetic substance and is obtained in the racemic or *d,l*-form. The problem of resolving glyceraldehyde and adding hydrogen cyanide to the optically active forms is extremely difficult. The Rosanoff classification is independent of the actual fulfillment of this step. However, this difficult work was finally accomplished by Wohl and Momber⁴⁰ (1914 and 1917), and (dextro)-glyceraldehyde was related to (levo)-tartaric acid. The same (levo)-tartaric acid had been obtained by Maquenne⁴¹ (1901) from the oxidation of the threose formed by the Wohl degradation of natural *d*-xylose, and thus the hexose

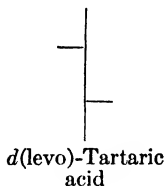
³⁹ Scheele, *cf.* Bugge (Lockemann), "Das Buch der grossen Chemiker," Verlag Chemie, Berlin (1929), Vol. I, p. 282.

* The rotatory sign (dextro) or (levo) refers to that of the equilibrated aqueous solution.

⁴⁰ Wohl and Momber, *Ber.*, **47**, 3346 (1914); **50**, 455 (1917).

⁴¹ Maquenne, *Ann. chim. phys.*, [7] **24**, 399 (1901).

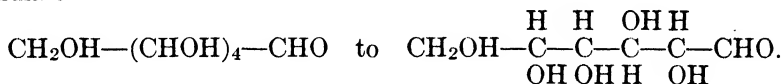
degradation methods met the cyanohydrin procedures at the tetrose stage. This leads to the designations *d*(dextro)-glyceraldehyde and *d*(levo)-tartaric acid. The assignment of the *d*-configuration to levorotatory tartaric acid is of course contrary to the general usage of the terms *d*- and *l*- for the tartaric acids, wherein these symbols are employed merely to denote the sign of rotation.



The results and significance of this work were incorporated in an important article by Wohl and K. Freudenberg⁴² (1923).

RING STRUCTURE AND TAUTOMERIC FORMS

Introduction. Returning again to the central compound, *d*-glucose, which serves as the prototype of all its relatives, it is found that the rather involved discussion just completed has advanced the Kiliani formula of



Fischer⁴³ (1893) next began to study further reactions of this polyhydroxy aldehyde, and one of the problems he attacked was that of its behavior, under acetal-forming conditions, with methanol and hydrogen chloride. He obtained no true acetal but instead only one methyl group entered the molecule. The product was non-reducing but showed reducing properties after acid hydrolysis. In the following year Alberda van Ekenstein⁴⁴ (1894) isolated a second isomer from the same reaction. To explain such results, Fischer adopted the ring-structure formula for these derivatives which had previously been suggested by Tollens⁴⁵ (1883) for *d*-glucose. At the same time Fischer correctly insisted that the facts as then known did not warrant the extension of this ring structure to *d*-glucose itself.

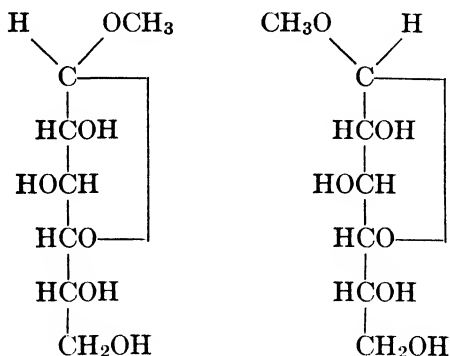
⁴² Wohl and Freudenberg, *Ber.*, **56**, 309 (1923).

⁴³ Fischer, *Ber.*, **26**, 2400 (1893).

⁴⁴ Alberda van Ekenstein, *Rec. trav. chim.*, **13**, 183 (1894).

⁴⁵ Tollens, *Ber.*, **16**, 921 (1883).

The two isomeric methanol condensation products of glucose were named by Fischer α -methyl-*d*-glucoside ($[\alpha]_D + 159^\circ$) and β -methyl-*d*-glucoside ($[\alpha]_D - 34^\circ$), and the structures assigned were:



These formulas represent inner cyclic acetals, and carbon one has become asymmetric, thus accounting for the two isomers. Being compounds of the acetal type, they are stable toward alkali but are hydrolyzed by acids. Fischer placed the ring closure on the fourth carbon, by analogy with the γ -lactones. This was an arbitrary assumption which later proved to be incorrect.

This classical work of Fischer confirmed previous indications that a ring-structure assignment was needed for other derivatives of glucose. Thus, Colley⁴⁶ (1870) had suggested a ring structure to explain the reactions of a crystalline acetoneglucose, and A. Michael⁴⁷ (1879) had synthesized a phenol glucoside. Skraup⁴⁸ (1889) had decided that glucose pentabenzoate contained no free aldehyde group, and finally Franchimont⁴⁹ (1879; 1892) and Erwig and Koenigs⁵⁰ (1889) had used this structure to explain the known isomeric pair of glucose pentaacetates.

Mutarotation. The extension to *d*-glucose of the ring structure clearly required by these methylglucosides involves a consideration of the phenomenon of mutarotation (p. 221). Dubrunfaut⁵¹ (1846) first observed that the optical rotation of a freshly prepared aqueous solution of glucose gradually fell to a constant value. This being about one-half of the original, he termed the phenomenon birotation. This property

⁴⁶ Colley, *Ann. chim. phys.*, [4] **21**, 363 (1870).

⁴⁷ Michael, *Am. Chem. J.*, **1**, 305 (1879).

⁴⁸ Skraup, *Monatsh.*, **10**, 401 (1889).

⁴⁹ Franchimont, *Ber.*, **12**, 1940 (1879); *Rec. trav. chim.*, **11**, 106 (1892).

⁵⁰ Erwig and Koenigs, *Ber.*, **22**, 2207 (1889).

⁵¹ Dubrunfaut, *Compt. rend.*, **23**, 38 (1846).

is possessed by all sugars, which reduce Fehling's solution, with the exception of some of the ketoses. However, the fall to a half value was, of course, only adventitious for glucose, and the name mutarotation as suggested by Lowry⁵² (1899) is now used. Fischer, the experimentalist, did not concern himself with this rotation change other than merely to suggest that perhaps it was due to hydration.

The experimental evidence required to interpret the mutarotation of glucose was furnished by Tanret⁵³ (1895) when he prepared two isomeric forms of *d*-glucose. One of these, α -*d*-glucose, shows a rotation change of $+113^\circ \rightarrow +52.5^\circ$; the other, β -*d*-glucose, $+19^\circ \rightarrow +52.5^\circ$. They are thus mutually interconvertible into an equilibrium mixture. Tanret obtained the equilibrium mixture as peculiar mixed crystals which he thought were homogeneous, but other workers soon corrected this error. When a sugar crystallizes from solution, it separates almost entirely in that form which is the least soluble under the conditions, the solution equilibrium then shifting to produce more of this isomer. The preparation of a sugar in its crystalline α - and β -forms thus becomes a difficult matter as it is necessary to find conditions under which each form will crystallize. Such conditions have been realized with only a few of the sugars. When the rotation of one form is known, the rotation of the other may be calculated by solubility relations according to a method developed by Hudson⁵⁴ (1904) and by Lowry⁵⁵ (1904). When α -*d*-glucose dissolves in water containing alcohol the solubility of this initial form, quickly attained, is measured. There then results a slow increase in solubility, appearing at a rate equal to that of the speed of mutarotation. The equilibrium solubility thus measures the combined concentrations of α - and β -forms, and the rotation of the β -form may be calculated. It is necessary, of course, to maintain an excess of the α -form in the solid phase in order to keep its solubility constant. This method depends upon the presence in the equilibrium mixture of only two forms in appreciable amount. When the method was applied to sugars which were accessible both in the α - and in the β -forms, the expected results were obtained. The β -isomer of *d*-mannose was the first known form of this sugar, and when Levene⁵⁶ (1923) succeeded in preparing the α -isomer, its rotation was in agreement with that calculated by Hudson and Yanovsky⁵⁷ (1917).

The kinetics of sugar mutarotation have been extensively studied

⁵² Lowry, *J. Chem. Soc.*, **75**, 211 (1899).

⁵³ Tanret, *Bull. soc. chim.*, [3] **13**, 728 (1895).

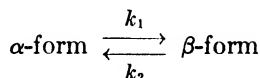
⁵⁴ Hudson, *J. Am. Chem. Soc.*, **26**, 1065 (1904).

⁵⁵ Lowry, *J. Chem. Soc.*, **85**, 1551 (1904).

⁵⁶ Levene, *J. Biol. Chem.*, **57**, 329 (1923); **59**, 129 (1924).

⁵⁷ Hudson and Yanovsky, *J. Am. Chem. Soc.*, **39**, 1013 (1917).

by many workers, among whom Hudson, Lowry, Osaka, and Riiber have been outstanding. The course of the optical rotation change in general follows the unimolecular law. An equation for calculating the velocity constant has been developed which does not require a knowledge of the molecular rotation of the second form.



$K = k_1 + k_2 = \frac{1}{t} \log \frac{r_0 - r_\infty}{r_t - r_\infty}$, wherein K is the resultant velocity constant; k_1 and k_2 are the velocity constants of the two opposing reactions; t = time; r_0 = initial rotation; r_∞ = final rotation; r_t = rotation at time t .

For *d*-glucose, Hudson and Dale⁵⁸ (1917) found the value $k_1 + k_2 = 0.00625$ in water at 20° (minutes and decimal logarithms). This constant is identical for both forms of the sugar. The velocity of mutarotation is greatly accelerated by acids and bases, and this point was studied thoroughly by Osaka and by Hudson. The correct relation for this effect was given by Hudson⁵⁹ (1907) for *d*-glucose in water in the form:

$$K = 0.0096 + 0.258 [\text{H}^+] + 9750 [\text{OH}^-]$$

This equation indicates that the acceleration of mutarotation by hydrogen and hydroxyl ions is directly proportional to their concentration and that the catalytic activity of hydroxyl ions is about 40,000 times as great as that of hydrogen ions. From the constant terms in the above equation and the velocity of mutarotation of glucose in pure water, Hudson⁶⁰ (1909) calculated the dissociation constant of water as 1.0×10^{-14} , which is in good agreement with the values obtained by other methods.

Lowry has shown that mutarotation is not effected without a catalyst and that both a proton donor and acceptor are required. He has also shown that the phenomenon of mutarotation is not confined to the sugar group and that only an amphoteric solvent, such as water, is a true catalyst for mutarotation. Lowry and Faulkner⁶¹ (1925) showed that the mutarotation of tetramethylglucose (p. 1422) could be arrested in a pyridine (weak base) solution and in a cresol (weak acid) solution, but a mixture of these two solvents gave a velocity twenty

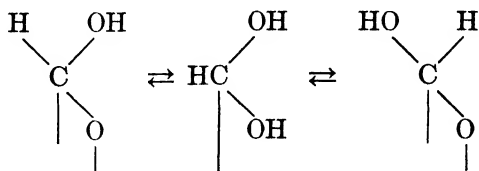
⁵⁸ Hudson and Dale, *ibid.*, **39**, 320 (1917).

⁵⁹ Hudson, *ibid.*, **29**, 1572 (1907).

⁶⁰ Hudson, *ibid.*, **31**, 1136 (1909).

⁶¹ Lowry and Faulkner, *J. Chem. Soc.*, **127**, 2883 (1925).

times as great as that of water. Lowry terms mutarotation a prototropic change and attributes it to a proton shift in which the solvent plays a part. He interprets the change of an α - to a β -sugar as passing through an intermediate acyclic form (in water, the aldehydrol).



The deviation from the monomolecular law of the first part of the galactose mutarotation curve lends itself to this interpretation, and Smith and Lowry⁶² (1928) have treated the data from the standpoint of a three-membered equilibrium. Evidence that more than two forms of a sugar are involved in mutarotation phenomena has also been obtained by Riiber⁶³ (1922 on) from studies of changes in volume and refractivity of sugar solutions undergoing mutarotation.

Finally, it may be stated that the problem of why the majority of the ketoses do not exhibit mutarotation is still unsolved.

α -, β -Isomerism. The preceding discussion of the phenomenon of mutarotation certainly establishes the fact that *d*-glucose, in common with all the mutarotating sugars, exists in at least two isomeric forms, the ordinary or α -*d*-glucose and the second or β -*d*-glucose. E. F. Armstrong⁶⁴ (1903) was able to relate these two forms of glucose to the two methylglucosides of Fischer by means of a very simple and beautiful experiment. Fischer⁶⁵ (1894) had found that α -methylglucoside was hydrolyzed by the enzyme maltase and the β -isomer by emulsin. Armstrong simply observed these enzymatic hydrolyses polarimetrically and established the fact that the α -glucoside liberated initially the higher rotatory form of glucose and the β - the lower or β -glucose. Behrend and Roth⁶⁶ (1904) also related α - and β -glucose to the two known glucose pentaacetates by acetylation with pyridine and acetic anhydride at 0°. α -Glucose produced α -glucose pentaacetate ($[\alpha]_D + 102^\circ$, CHCl_3) and β -glucose yielded the β -pentaacetate ($[\alpha]_D + 4^\circ$, CHCl_3). Thus the presence of a ring structure in *d*-glucose was established. There remained the problem of establishing upon good experi-

⁶² Smith and Lowry, *ibid.*, 666 (1928).

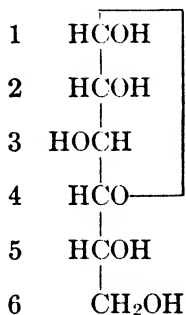
⁶³ Riiber, *Tids. Kjem. Bergvesen*, **12**, 227 (1932) [*C.A.*, **27**, 958 (1933)], summarizing paper.

⁶⁴ Armstrong, *J. Chem. Soc.*, **83**, 1305 (1903).

⁶⁵ Fischer, *Ber.*, **27**, 2985 (1894).

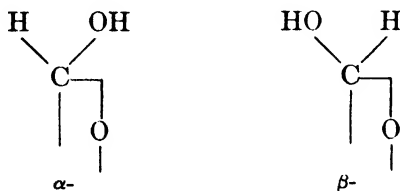
⁶⁶ Behrend and Roth, *Ann.*, **331**, 359 (1904).

mental evidence two points: first, the point of ring closure; and second, the configuration of carbon one. The first was soon forgotten and was revived only much later. The second point is very difficult and has not been satisfactorily solved at the present date. Böeseken⁶⁷ (1913) made an attempt to solve this question of the space position of the groups attached to carbon one. This was based upon his observations regarding the effect of the constitution of hydroxy compounds on the electrical conductivity of boric acid solutions—namely, that a *cis* configuration in a glycol produced a complex with boric acid which was a stronger acid than that produced by the *trans* isomer. The conductivity of α -glucose in the presence of boric acid decreases during mutarotation as it is converted in part into β -glucose; the reverse is true of β -glucose. The velocity of this change parallels that of the mutarotation. Accordingly α -glucose was given the formula:



The numbering in the above formula indicates the customary method of designating the individual carbon atoms of a monosaccharide, the numbering beginning from the top or reducing portion of the molecule. With a ketose the carbonyl carbon is carbon two.

Hudson⁶⁸ (1909) has given an empirical rule for designating α -, β -isomers. Of an α -, β -pair of sugars in the *d*-series, he terms the α - that one which has the higher dextro rotation and assigns the hydroxyl to the right.



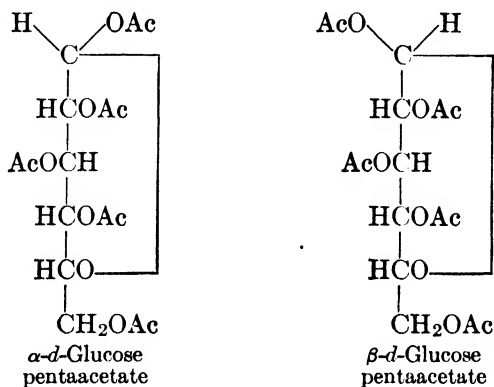
The reverse of course holds for the *l*-series, the enantiomorph of α -*d*-

⁶⁷ Böeseken, *Ber.*, **46**, 2612 (1913).

⁶⁸ Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909).

glucose being designated α -*l*-glucose. There is a need for the determination of the configuration of carbon one in the sugars by direct chemical methods, and the indications are that this may soon be accomplished by the application of new oxidizing agents to the sugar series.

The knowledge of the α -, β -isomerism of sugars and their derivatives which is now available is due mainly to the work of Hudson and his co-workers. Hudson and Yanovsky⁶⁹ (1917) measured the rotation values of the unknown forms of the sugars by the maximum solubility method, but Hudson turned to the acetates of the sugars for a more convenient and richer source of pure α -, β -isomers. The dextro or α -glucose pentaacetate had been prepared by Franchimont⁷⁰ (1879; 1892), and the β - by Erwig and Koenigs⁷¹ (1889). They have the following structures, in which the ring assignment was demonstrated later by methods yet to be described.



The methylglycosides (glycose referring to any sugar) and their acetates were also included in Hudson's studies.

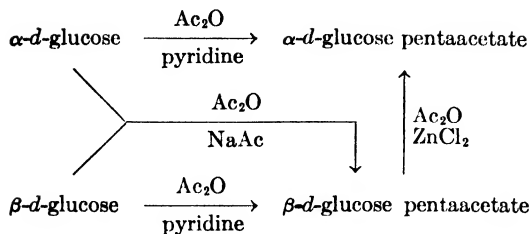
It will be of interest to describe briefly the methods used in obtaining isomeric sugar acetates. When the two crystalline forms of a sugar are known, the previously cited Behrend acetylation at 0° with pyridine and acetic anhydride serves admirably.⁷² The β -isomer (for the *d*-series) is obtained by acetylation of either form of the free sugar with hot acetic anhydride and sodium acetate. The β -isomer is transformed to the α -form by heating with acetic anhydride and zinc chloride, a reaction due to Erwig and Koenigs⁷¹ (1889) but correctly interpreted and greatly extended by Hudson.

⁶⁹ Hudson and Yanovsky, *ibid.*, **39**, 1013 (1917).

⁷⁰ Franchimont, *Ber.*, **12**, 1940 (1879); *Rec. trav. chim.*, **11**, 106 (1892).

⁷¹ Erwig and Koenigs, *Ber.*, **22**, 2207 (1889).

⁷² Hudson and Dale, *J. Am. Chem. Soc.*, **37**, 1267 (1915).



Rules of Optical Rotation. Hudson became greatly interested in a study of the numerical values of optical rotations, being especially concerned with testing the van't Hoff theory of optical superposition in the sugar group. He⁷³ (1909) developed two rules which he termed the rules of isorotation. If the rotation contributed by carbon one is termed A and that of the remaining asymmetric centers B, then the molecular rotations will be:

$$\begin{array}{ll}
 \text{for the } \alpha\text{-form (d-series),} & A + B \\
 \text{" " } \beta\text{-form " " ,} & -A + B
 \end{array}$$

It follows from the above that in an α -, β - pair of isomers, the sum (2B) of their molecular rotations will be a constant (rule 1) characteristic of the particular sugar, and the difference (2A) will be a constant (rule 2) characteristic of the nature of the hydroxyl group or substituted hydroxyl on carbon one. These modified rules of optical superposition apparently do not have a rigid general application but hold remarkably well with many closely related structures, as is illustrated in Tables I, II, and III (pp. 1420 and 1441).

Having briefly discussed Hudson's rules of isorotation, a number of other rules based upon optical properties may be cited. Hudson⁷⁴ (1910) observed that, in the ordinary γ -lactones of the aldonic acids, the sign of rotation of the lactone was determined by the spatial configuration of the asymmetric center (carbon four) where lactonization took place. If carbon four was (+), hydroxyl on the right, then the lactone was dextrorotatory; and if carbon four was (-), hydroxyl on the left, then the lactone showed a levorotation. Levene⁷⁵ (1915) obtained evidence to show that when carbon two in a sugar acid is (+), then the ion (rotation of the salt) is more dextrorotatory than the slightly dissociated free acid, and *vice versa*. It was also noted that the sign of rotation of the phenylhydrazides of the sugar acids was determined by the sign of carbon two: when this was (+) the hydrazide was dextroro-

⁷³ Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909).

⁷⁴ Hudson, *ibid.*, **32**, 338 (1910).

⁷⁵ Levene, *J. Biol. Chem.*, **23**, 145 (1915).

TABLE I
OPTICAL ROTATIONS OF ACETYLATED SUGARS IN CHLOROFORM

Substance	Molecular Rotation of α -form	Molecular Rotation of β -form	2A (Differ- ence)	2B (Sum)
<i>l</i> -Arabinose tetraacetate *	+13,500°	+46,800°	-33,300	+60,300
<i>d</i> -Xylose tetraacetate	+28,300	-7,900	+36,200	+20,400
<i>d</i> -Glucose pentaacetate	+39,600	+1,500	+38,100	+41,100
<i>d</i> -Mannose pentaacetate	+21,500	-9,800	+31,300	+11,700
<i>d</i> -Galactose pentaacetate	+41,700	+9,000	+32,700	+50,700
<i>d</i> -[α -Glucoheptose] hexaacetate	+40,200	+2,200	+38,000	+42,400
<i>d</i> -Glucosamine pentaacetate	+36,400	+470	+35,930	+36,900
<i>d</i> -Chondrosamine pentaacetate	+39,400	+4,100	+35,300	+43,500
Cellobiose octaacetate	+27,800	-9,900	+37,700	+17,900
Gentiobiose octaacetate	+35,500	-3,600	+39,100	+31,900
Lactose octaacetate	+36,500	-2,900	+39,400	+33,600
Maltose octaacetate	+83,000	+42,500	+40,500	+125,500

* The negative sign of 2A is due to this being an *l*-sugar.

TABLE II
OPTICAL ROTATIONS OF ACETYLATED METHYLGLYCOSIDES IN CHLOROFORM

Substance	Molecular Rotation of α -form	Molecular Rotation of β -form	2A (Differ- ence)
Methyl- <i>d</i> -xyloside triacetate	+34,700°	-17,600°	+52,300
Methyl- <i>d</i> -glucoside tetraacetate	+47,300	-6,600	+53,900
Methyl- <i>d</i> -galactoside tetraacetate	+48,400	-5,100	+53,500
Methylgentiobioside heptaacetate	+41,900	-12,350	+54,250
Methylcellobioside heptaacetate	+36,200	-16,500	+52,700
Methyl- <i>d</i> -guloside tetraacetate	+35,200	-11,600	+46,800
Methyl- <i>d</i> -[α -glucoheptoside] pentaacetate	+39,500	-6,900	+46,400
Methyl- <i>d</i> -mannoside tetraacetate	+17,800	-18,100	+35,900
Methyl- <i>l</i> -rhamnoside triacetate *	-16,300	+13,900	-30,200

* Measured in acetylene tetrachloride solution.

tatory, and *vice versa*. This is known as the hydrazide rule of Levene and Hudson. Hudson⁷⁶ (1918) found that it applies likewise to the sugar amides, and Deulofeu⁷⁷ (1933) has shown that it also applies to the

⁷⁶ Hudson, *J. Am. Chem. Soc.*, **40**, 813 (1918).

⁷⁷ Deulofeu, *Nature*, **131**, 548 (1933).

acetylated nitriles of the sugar acids. These rules are of a more qualitative nature than the isorotation rules and have found wide application. In particular, the lactone rule of Hudson has been of great value. For example, Clark⁷⁸ (1922) determined the configuration of carbon five in the methylpentose *L*-fucose (p. 1450) by a clever application of this rule. Anderson⁷⁹ (1912) has shown that β -*D*-metasaccharonolactone (p. 1516) rotates slightly to the left (-4.7°) and is in disagreement, therefore with the relation between rotation and structure because its γ -ring is to the right of the structure. As Anderson points out, however, β -saccharonic acid is strongly levorotatory, and the change of rotation due to lactone formation is in the direction called for by theory. The same explanation probably holds for the small levorotation of *D*-allonolactone (-6.8°), which is in the opposite direction to that indicated by theory.

Establishment of the Pyranose Ring Structure. In 1915-1916 Hudson⁸⁰ and his co-workers Parker and Johnson were investigating the α - and β -pentaacetates of *D*-galactose and obtained four crystalline isomers, corresponding with two α -, β -pairs. This represented excellent evidence, based upon crystalline derivatives, that more than one ring form could exist in a sugar and could be explained on the basis of ring closure on different carbon atoms. Similar compounds were not obtained in the glucose series until 1927 when Schlubach and Huntenburg⁸¹ added the third and fourth pentabenzates of *D*-glucose to the two previously prepared by Skraup⁸² (1889) and by Fischer and co-workers⁸³ (1911; 1912). Sugar benzoates are rather difficult to purify, and Levene and Meyer⁸⁴ (1928) were able to change considerably the constants given by Fischer and by Schlubach for these compounds.

The determination of the size of the oxide ring in sugar derivatives has been accomplished by means of methylation studies. Purdie had developed a workable method for obtaining methyl ethers of hydroxy acids, which consisted in reacting the alcoholic substance with methyl iodide and silver oxide. In 1903 Purdie and Irvine⁸⁵ published the results of their extension of this reaction to α -methyl-*D*-glucoside. A pentamethyl derivative was obtained which could be distilled in a good

⁷⁸ Clark, *J. Biol. Chem.*, **54**, 65 (1922).

⁷⁹ Anderson, *J. Am. Chem. Soc.*, **34**, 51 (1912).

⁸⁰ Hudson and Parker, *ibid.*, **37**, 1589 (1915); Hudson and J. M. Johnson, *ibid.*, **38**, 1223 (1916).

⁸¹ Schlubach and Huntenburg, *Ber.*, **60**, 1487 (1927).

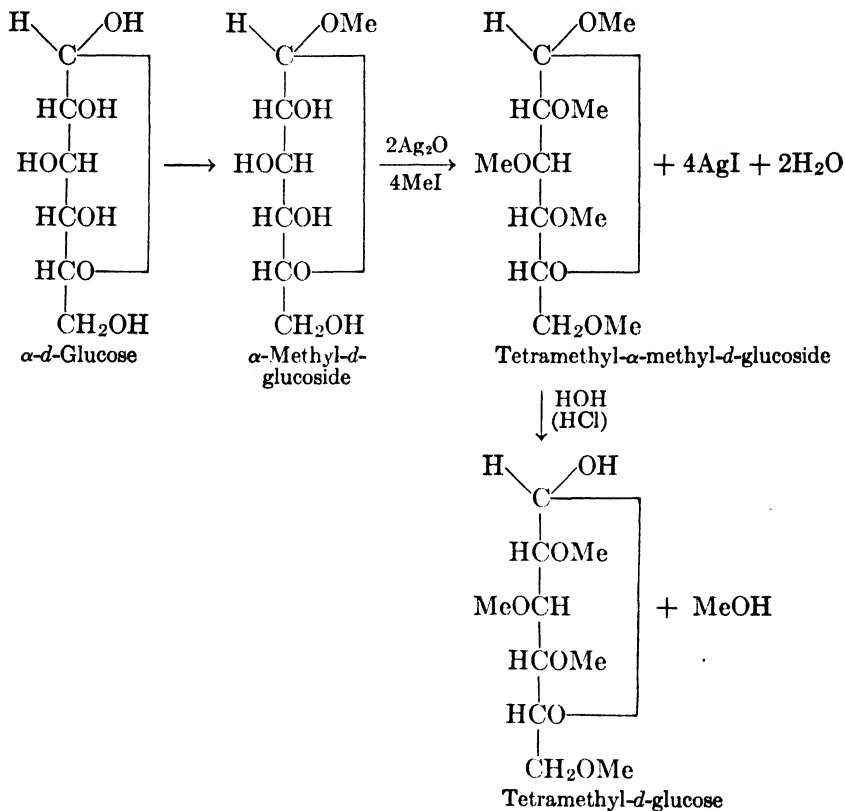
⁸² Skraup, *Monatsh.*, **10**, 395 (1889).

⁸³ Fischer and Helferich, *Ann.*, **383**, 68 (1911); Fischer and K. Freudenberg, *Ber.* **45**, 2724 (1912).

⁸⁴ Levene and Meyer, *J. Biol. Chem.*, **76**, 513 (1928).

⁸⁵ Purdie and Irvine, *J. Chem. Soc.*, **83**, 1021 (1903).

vacuum and which on hydrolysis lost the glycosidic (carbon one) methyl group and produced a crystalline tetramethylglucose. The latter fact was indeed fortunate, as this substance still remains one of the few methylated sugars which crystallizes with any ease. In this derivative the hydroxyl groups are blocked with stable ether groups, and many results could now be obtained which were not possible with substituents less resistant to chemical action.



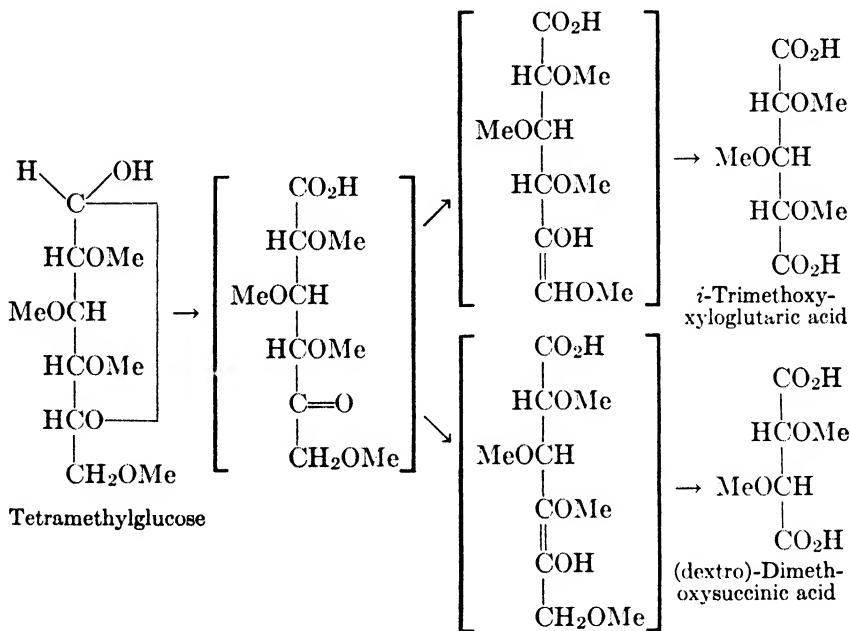
An alternative methylation procedure applicable to the sugar series is that employing methyl sulfate and alkali. This general method was perhaps first recorded in the scientific journals by Pschorr and Dickhäuser⁸⁶ (1911); it was applied to the methylation of cellulose by Denham and Woodhouse⁸⁷ (1913); and was shown to be suitable for the methylation of glycosides by Haworth⁸⁸ (1915).

⁸⁶ Pschorr and Dickhäuser, *Ber.*, **44**, 2633 (1911).

⁸⁷ Denham and Woodhouse, *J. Chem. Soc.*, **103**, 1735 (1913).

⁸⁸ Haworth, *ibid.*, **107**, 13 (1915).

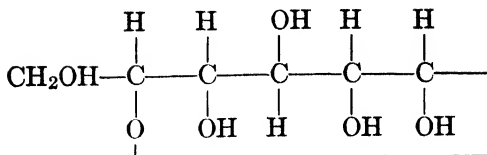
Tetramethylglucose is a substance which can be oxidized, and from a study of its oxidation products the point of ring closure may be ascertained. Hirst⁸⁹ (1926) oxidized tetramethylglucose with nitric acid and identified the acids formed by means of their crystalline diamides. He identified *i*-trimethoxyxyloglutaric acid and (dextro)-dimethoxysuccinic acid among the oxidation products and thus established the fact that the ring closure in tetramethylglucose was on carbon five. The diamide of this methylated tartaric acid had been previously characterized by Purdie and Irvine⁹⁰ (1901).



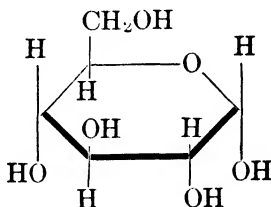
Since Armstrong had related α -*D*-glucose to α -methylglucoside, then α -glucose itself has the same ring structure as tetramethylglucose, provided no ring shift occurred during the methylation process. Since β -methylglucoside likewise yields the same tetramethylglucose, then α - and β -glucose have the same ring structure. This is variously denoted as (1,5-), amylenic, oxidic, normal or pyranose. The last name was suggested by Haworth and is preferable. Accordingly, the ordinary crystalline form of glucose is accurately named α -*D*-glucopyranose and has the following structure:

⁸⁹ Hirst, *ibid.*, 350 (1926).

⁹⁰ Purdie and Irvine, *ibid.*, 79, 960 (1901).



Haworth correctly considers that the true spatial relationships are better shown by a hexagonal formula, and this type of representation has been widely adopted, especially for depicting the disaccharide and polysaccharide molecules. Cox has obtained x-ray evidence in support of such a ring.



Establishment of the Furanose Ring Structure. The pyranose ring structure established for α - and β -*D*-glucose has been extended to other sugars, and it has been determined that the normal or ordinary forms of the sugars and their derivatives possess the pyranose ring. Other ring structures are possible, however. In 1932 Haworth⁹¹ reported the synthesis of a third crystalline methylglucoside which contained a (1,4-) or furanose ring. Substances containing this unstable ring have also been termed butylene oxidic or γ -sugars. In place of the name ring, the term lactol has been suggested by Helferich, this name being analogous to lactone. As the above glucofuranoside was synthesized from monoacetoneglucose, it is necessary to digress sufficiently to discuss the structure of this ketone condensation product of glucose. In working with the sugar alcohols, Meunier⁹² (1888) had characterized them as their benzal derivatives, in which the benzaldehyde had undergone acetal formation with the polyhydroxy sugar alcohol to form a cyclic acetal. This reaction is general for polyhydroxy compounds and is effected by treatment of the substance with the aldehyde or ketone in the presence of a dehydrating agent such as zinc chloride or sulfuric acid. In 1895 Fischer⁹³ obtained a crystalline derivative of glucose in which two moles of acetone had reacted with the glucose. This substance is known as diacetoneglucose, and on graded acid hydrolysis,

⁹¹ Haworth, Porter, and Waine, *ibid.*, 2254 (1932).

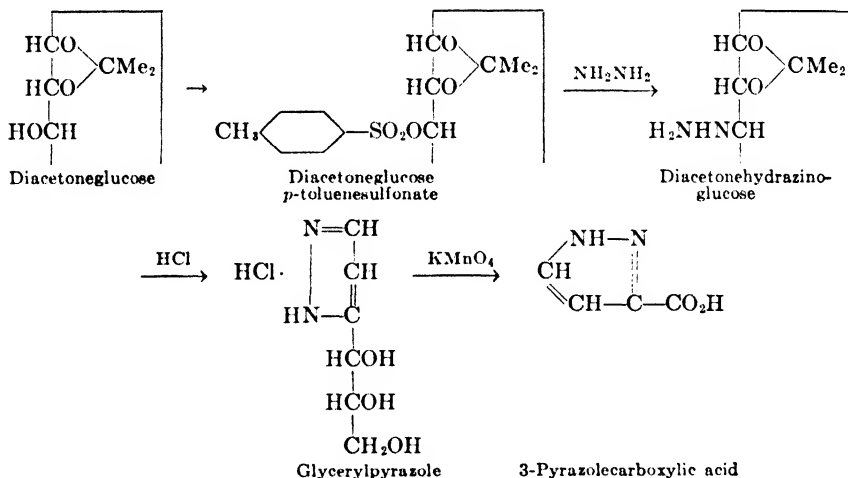
⁹² Meunier, *Compt. rend.*, **106**, 1425 (1888).

⁹³ Fischer, *Ber.*, **28**, 1165 (1895).

crystalline monoacetoneglucose is formed. The structure of these two compounds has been the subject of a number of investigations.

Irvine and Scott⁹⁴ (1913) methylated diacetoneglucose, and after removal of the acetone groups they obtained a beautifully crystalline monomethylglucose which formed a crystalline monomethyl phenylosazone. They also obtained a syrupy trimethylglucose from monoacetoneglucose. Neither of the two unsubstituted acetoneglucoses reduces Fehling's solution. These facts place the acetone group of monoacetoneglucose on positions one and two. Levene and Meyer⁹⁵ (1922) oxidized this monomethylglucose with nitric acid and obtained a crystalline monomethylglucosaccharolactone, thus eliminating positions one and six for the methyl substituent.

The allocation of position three for the open hydroxyl of diacetoneglucose was made by K. Freudenberg and by Levene through the use of entirely different methods of proof. K. Freudenberg and Doser⁹⁶ (1923) converted diacetoneglucose to the previously known 3-pyrazolecarboxylic acid through the following steps, all products being crystalline.



Levene and Meyer⁹⁷ (1924) converted the monomethylglucose of Irvine and Scott to a crystalline monomethylglucoheptonolactone through the cyanohydrin reaction. This lactone was dextrorotatory (+48°) whereas the lactone of *d*-α-glucuheptonic acid is levorotatory (-56°). Therefore, in accordance with Hudson's lactone rule, the

⁹⁴ Irvine and Scott, *J. Chem. Soc.*, **103**, 570 (1913).

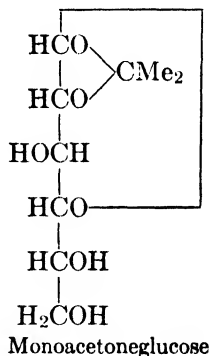
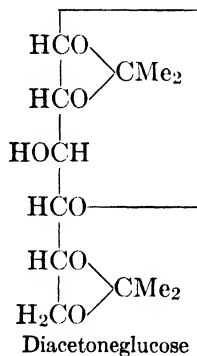
⁹⁵ Levene and Meyer, *J. Biol. Chem.*, **54**, 805 (1922).

⁹⁶ Freudenberg and Doser, *Ber.*, **56**, 1243 (1923).

⁹⁷ Levene and Meyer, *J. Biol. Chem.*, **60**, 173 (1924).

single (—) carbon (hydroxyl on left) of glucose is occupied by a methoxyl group in the monomethylglucose, and this (—) carbon atom is known to be number three. Levene and Simms⁹⁸ (1925) showed that the above 4-methylglucoheptonolactone was an unstable or δ -lactone.

The above work gives the structure of the first three carbons of diacetoneglucose, but does not establish the nature of the remainder. Levene and Meyer⁹⁹ (1926), and also Micheel and Hess¹⁰⁰ (1926), further methylated the syrupy trimethylglucose obtained from monoacetoneglucose and obtained a ring isomer of tetramethylglucopyranose. The furanose nature of this ring was proved definitely by Anderson, Charlton, and Haworth¹⁰¹ (1929) by oxidation to 2,3,5,6-tetramethylgluconic acid, isolated as its crystalline γ -lactone and crystalline phenylhydrazide. The fact that the acetone group of monoacetoneglucose is placed on carbon atoms one and two was established beyond doubt by the isolation of a crystalline trimethylglucose phenylosazone of the trimethylglucose by these workers. Accordingly, it is now apparent that in diacetoneglucose, positions one and two carry an acetone group; three is open, and the lactol ring is on carbon four. This leaves positions five and six for the second acetone group. It is of interest that a furanose (1,4-) derivative is thus directly obtained from an acid solution of glucose.



The above proof of structure does not involve the unwarranted assumption that acetone reacts only with hydroxyl groups that are adjacent. This assumption has been definitely disproved by the thorough studies of Hibbert and co-workers on glycerol cyclic acetals. He has shown that a partition is established between the five- and six-membered cyclic acetals. This variation in ring size has a bearing on

⁹⁸ Levene and Simms, *ibid.*, **65**, 31 (1925).

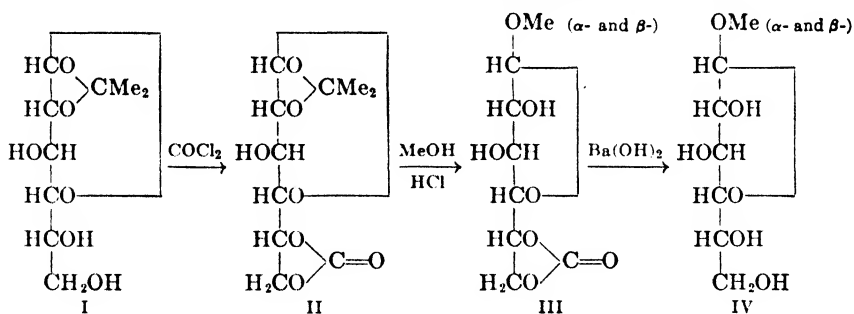
⁹⁹ Levene and Meyer, *ibid.*, **70**, 343 (1926).

¹⁰⁰ Micheel and Hess, *Ann.*, **450**, 21 (1926).

¹⁰¹ Anderson, Charlton, and Haworth, *J. Chem. Soc.*, 1329 (1929).

sugar lactol structure, as sugar lactols are really five- and six-membered cyclic hemiacetals.

Monoacetoneglucose is a non-reducing structure in which the furanose ring is stabilized. By reacting this substance (I) with phosgene, Haworth and Porter¹⁰² (1929) obtained a crystalline 5,6-carbonate of monoacetoneglucose (II). The structure of this substance was proved by converting its *p*-toluenesulfonate into the known *p*-toluenesulfonate of diacetoneglucose. In this mixed carbonate and cyclic acetal of glucose, Haworth possessed a compound wherein the acetone group was sensitive to acidity and stable to alkali, whereas the carbonate ester group had the reverse reactivity. Reaction of this substance (II) with methanol and hydrogen chloride resulted in the loss of the acetone group and formation of the crystalline α - and β -methylglucofuranoside-5,6-carbonates (III), the furanose ring structure being meanwhile stabilized by the carbonate group. These were separated, and mild saponification produced the glucofuranosides (IV), of which only the α -form was obtained crystalline. This work was completed by Haworth, Porter, and Waine¹⁰³ in 1932. In the case of the similar ethylglucofuranosides, Haworth and Porter¹⁰⁴ (1929) succeeded in obtaining both α - and β -forms in crystalline condition, the separation being effected through fractionation of their crystalline 5,6-carbonate-2,3-diacetates.



These furanosides are characterized by their ease of hydrolysis with acids, and the ring is accordingly very labile. They are not affected by dilute permanganate or by Fehling's solution. Previous statements that such behavior was characteristic of γ -glycosides were thus shown to be in error, easily oxidizable impurities being present in the older syrupy preparations.

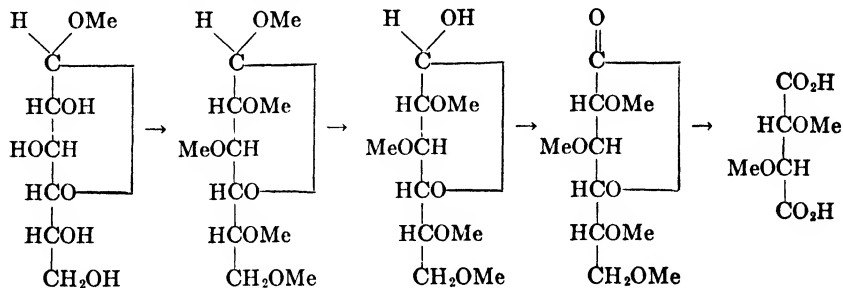
Complete methylation of α -methylglucofuranoside followed by hydrolysis of the glycosidic methyl group produces the syrupy tetra-

¹⁰² Haworth and Porter, *ibid.*, 2796 (1929).

¹⁰³ Haworth, Porter, and Waine, *ibid.*, 2254 (1932).

¹⁰⁴ Haworth and Porter, *ibid.*, 2796 (1929).

methylglucofuranose or γ -tetramethylglucose. This is a poorly characterized substance, but on oxidation with hypobromite it nevertheless forms a crystalline lactone, which in turn may be converted to a crystalline phenylhydrazide. Nitric acid oxidation of this lactone by Haworth, Hirst, and Miller¹⁰⁵ (1927) yielded (dextro)-dimethoxysuccinic acid, isolated as the crystalline amide and methylamide.



The α - and β -glucofuranose pentabenzoates of Schlubach are well-characterized crystalline derivatives of glucofuranose. Their structure follows from their method of preparation from monoacetoneglucose, in which the furanose ring is established by the experiments previously cited. Fischer and Rund¹⁰⁶ (1916) had benzoylated monoacetoneglucose and selectively hydrolyzed the acetone group with hydrochloric acid, thus producing the tribenzoate, which was isolated as a crystalline carbon tetrachloride addition compound. Fischer was merely interested in obtaining a partially benzoylated sugar and of course was unaware that he had in hand a glucose derivative containing an unusual ring. The contribution of Schlubach¹⁰⁷ (1927) was to benzoylate this substance further and to separate and isolate the two isomeric pentabenzoates thus formed.

The methods used for obtaining benzoylated sugars may be briefly mentioned. By means of the Schotten-Baumann method using dilute alkali and benzoyl chloride, Kueny¹⁰⁸ (1890) and other workers obtained some of the first acylated sugars. The difficulty with this method when applied to the sugars was that mixtures of partially benzoylated structures were generally formed. To obtain complete benzoylation of a sugar, Fischer¹⁰⁹ (1912) used successfully benzoyl chloride and quinoline. This procedure was later improved by substituting pyridine for the quinoline.

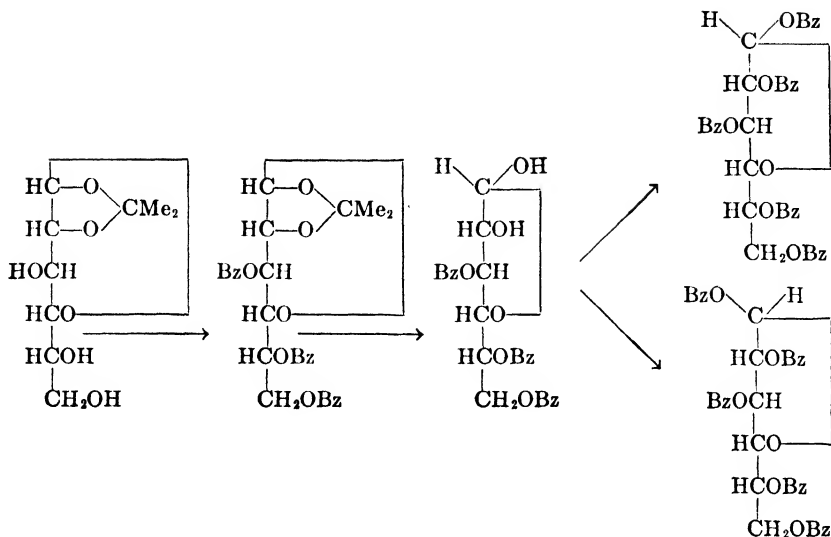
¹⁰⁵ Haworth, Hirst, and Miller, *ibid.*, 2436 (1927).

¹⁰⁶ Fischer and Rund, *Ber.*, **49**, 100 (1916).

¹⁰⁷ Schlubach and Hundenburg, *Ber.*, **60**, 1487 (1927).

¹⁰⁸ Kueny, *Z. physiol. Chem.*, **14**, 333 (1890).

¹⁰⁹ Fischer and K. Freudenberg, *Ber.*, **45**, 2724 (1912).



Tetramethylglucofuranose had been prepared in 1915 by Irvine¹¹⁰ and his students through methylation and subsequent hydrolysis of the so-called γ -methylglucoside obtained by Fischer¹¹¹ in 1914 and recognized by him as a ring isomer of the ordinary or normal methylglucosides. This γ - (called by the German workers *h*- for *hetero*) methylglucoside is the impure syrupy mixture which arises when glucose is allowed to react with methanol at room temperature in the presence of a considerable concentration of hydrogen chloride. This reaction is a general one for the reducing monosaccharides and the γ -glycosides so obtained are all characterized by their ease of hydrolysis. Some of the normal glycosides are likewise formed in the reaction, and Levene, Raymond, and Dillon¹¹² (1932) have obtained data to show that the γ -glycosides are produced initially and then rearrange in part, under the experimental conditions, to form the more stable pyranosides. It is probable that this is not a true rearrangement but is a hydrolysis followed by glycopyranoside formation. Very few crystalline isomers have been isolated from these γ -methylglycoside syrups. Purves and Hudson¹¹³ (1934) have isolated a crystalline isomer from the reaction product with fructose, and Haworth¹¹⁴ (1930) obtained crystalline α -methylmannofuranoside from mannose after he had obtained nuclei by an extension to man-

¹¹⁰ Irvine, Fyfe, and Hogg, *J. Chem. Soc.*, **107**, 524 (1915).

¹¹¹ Fischer, *Ber.*, **47**, 1982 (1914).

¹¹² Levene, Raymond, and Dillon, *J. Biol. Chem.*, **95**, 699 (1932).

¹¹³ Purves and Hudson, *J. Am. Chem. Soc.*, **56**, 708 (1934).

¹¹⁴ Haworth, Hirst, and Webb, *J. Chem. Soc.*, 651 (1930).

nose¹¹⁵ of his carbonate work, so successfully used in obtaining the pure methylglucofuranoside. A fortunate point with mannose is that this sugar tends to form only one glycoside, the α -, and so the number of possible isomers present in the syrupy γ -methylmannoside was accordingly decreased. All the methylated furanose sugars so far obtained through these γ -glycosides have been syrups. In the partially substituted sugar series, a crystalline 5-methyl-*L*-rhamnofuranose has been prepared.^{115a}

Lactone Studies Related to the Determination of Sugar Ring Structure. Any discussion of ring structure in the sugar series would be incomplete without a consideration of the supporting evidence obtained from the study of lactones. It has been mentioned how the early known γ -lactones of the sugar acids were important in synthetic work and also how a statistical study of their rotatory power led to the establishment of the lactone rule of Hudson. Some confusion entered this field when Nef and Hedenburg¹¹⁶ in 1914 isolated a *second* crystalline lactone of gluconic acid and also a *second* of mannonic acid. It was obvious that one lactone in each of the two pairs was not a γ -lactone. Of the two lactones, one was much more unstable than the other, and this unstable form was apparently the one which was not the γ -lactone.

In 1925¹¹⁷ Levene and Simms published a very important paper in which they showed clearly that, when a free aldonic acid was liberated from an aqueous solution of its salt by the addition of one equivalent of mineral acid, lactonization took place in two stages. The first was a very rapid formation of an unstable lactone, followed by the slow formation of the stable γ -lactone and the disappearance of the unstable lactone. The final equilibrium mixture apparently contained the γ -lactone in equilibrium with the free acid. This reminds one of the results obtained later by Levene, Raymond, and Dillon in their studies of methylglycoside formation.

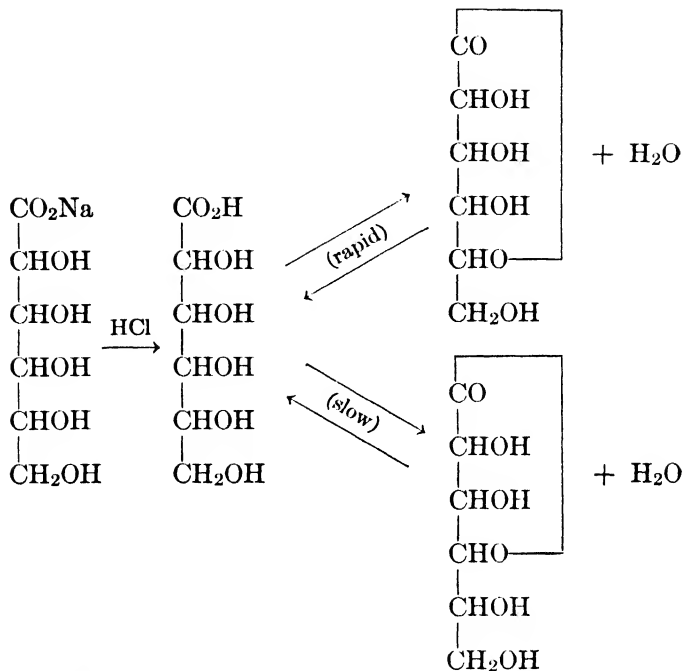
Levene and Simms¹¹⁷ (1925) applied this procedure to the two methylated mannonic acids. The one acid (I) was obtained from the crystalline methylation product of the stable mannonic lactone and the other (II) by oxidation of normal tetramethylmannose. Acid II rapidly formed an unstable lactone in aqueous solution; acid I showed the slow formation of a stable, apparently (1,4-), lactone. The conclusion was reached then that normal tetramethylmannose and thus also the ordinary α -methylmannoside did not possess a (1,4-) ring but probably had

¹¹⁵ Haworth and Porter, *ibid.*, 649 (1930).

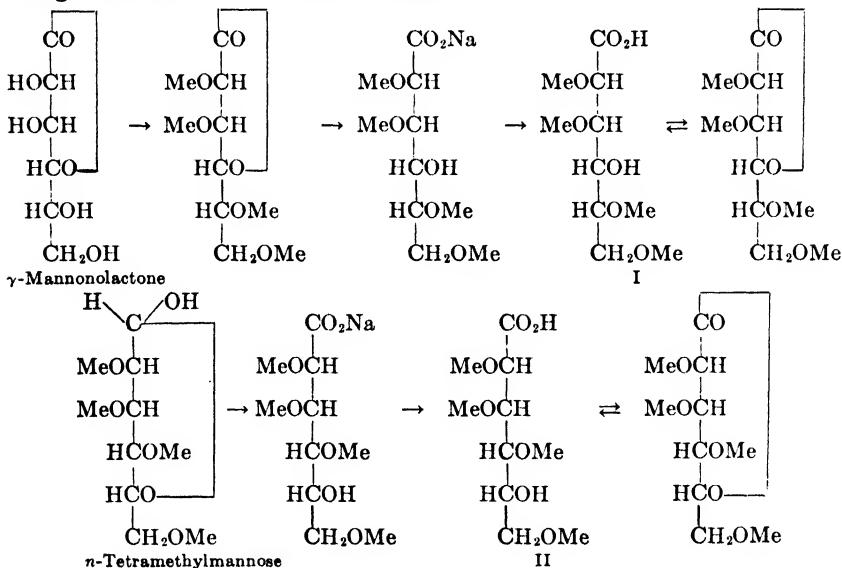
^{115a} Levene and Compton, *J. Biol. Chem.*, **114**, 9 (1936).

¹¹⁶ Nef, *Ann.*, **403**, 322 (1914); Hedenburg, *J. Am. Chem. Soc.*, **37**, 345 (1915).

¹¹⁷ Levene and Simms, *J. Biol. Chem.*, **65**, 31 (1925).

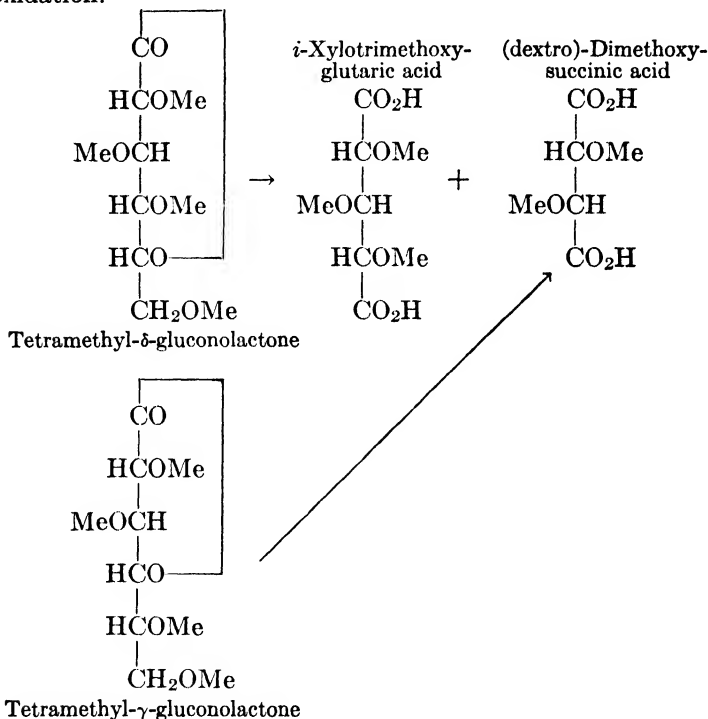


a (1,5-) ring. In 1926,¹¹⁸ Levene and Simms extended this work to the glucose series with similar results.



¹¹⁸ Levene and Simms, *ibid.*, 68, 737 (1926).

There had now been obtained by Haworth and by Irvine two series of methylated reducing sugars. The first or normal forms were those obtained from the ordinary methylglycosides, and the oxidation evidence gradually accumulated to show that all had the pyranose structure. The other series of methylated sugars was the one obtained from the Fischer γ -methylglycosides and contained the furanose (1,4-) ring. When the methylated sugars of either series were oxidized, the lactones of the corresponding methylated aldonic acids could be obtained. Of the two tetramethylgluconolactones, the γ - (1,4-) is crystalline and the δ - (1,5-) forms a crystalline phenylhydrazide. Their structures have also been determined by Haworth, Hirst, and Miller¹¹⁹ (1927) by nitric acid oxidation.



Haworth and his students¹²⁰ (1926) studied the rate of lactone hydrolysis exhibited by the methylated aldonolactones. This was the reverse process of the one studied by Levene and Simms. The results showed that the lactones obtained from the methylated aldoses of the γ -methylglycoside series hydrolyzed very slowly and exhibited all the properties of γ -lactones. On the other hand, the (1,5-) or δ -lactones

¹¹⁹ Haworth, Hirst, and Miller, *J. Chem. Soc.*, 2436 (1927).

¹²⁰ Charlton, Haworth, and Peat, *ibid.*, 89 (1926).

obtained from the ordinary or normal methylglycoside series showed a high speed of hydrolysis. The behavior toward hydrolysis of the (1,4-) and (1,5-) oxygen rings in the methylated lactones of the aldonic acids is thus the reverse of that exhibited by the corresponding oxygen rings of like size when present in a methylglycoside structure.

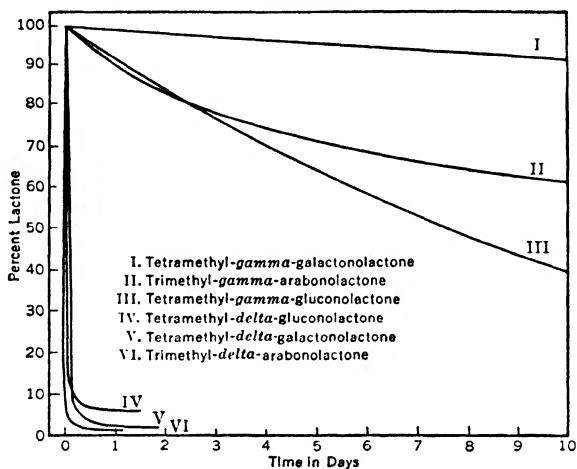
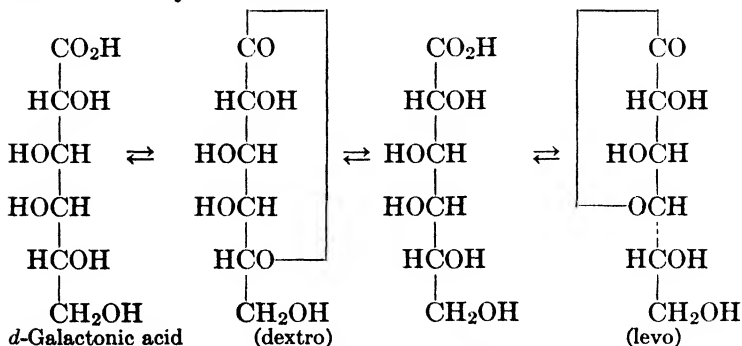


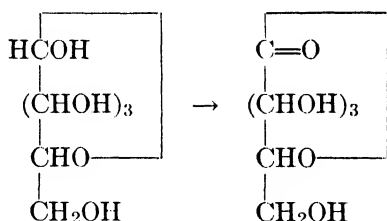
Fig. 2.*—Rates of hydrolysis of methylated lactones

It is inferred that the unstable lactones of the unmethylated aldonic acids noted by Nef and Hedenburg and by Levene and Simms are also δ -lactones. This inference is somewhat dangerous, as it involves reasoning by analogy. It is supported, however, by the optical data obtained by Levene and Simms for galactonic acid, in which a dextrorotatory lactone was formed first, followed by a levorotatory lactone. Now carbon atom five of *d*-galactonic acid is (+) and number four is (−), so that, if Hudson's rule may be extended to δ -lactones, the results are in harmony with a δ -structure.



* From Haworth, "The Constitution of Sugars," Arnold and Co., London (1929).
 (Courtesy of the publishers.)

A further use has been made of the divergent properties of the two types of aldonolactones in the direct determination of aldose ring structure. We have seen how the results of Armstrong allowed the pyranose structure of the normal methylglucosides to be extended to glucose itself. This proof was practically unique for *d*-glucose because of the specificity of enzymic action. It was tacitly assumed that the pyranose structures obtained by methylation of the methylglycosides could be extended to the free sugars. This was somewhat obscured by the fact that the "normal" methylglycoside was the one produced by the apparently more vigorous methylglycosidic formation conditions, as has been noted. There was a need, then, for a more direct determination of the ring structures of the reducing sugars. This has now been given by the studies initiated by Hudson and Isbell¹²¹ (1932), and elaborated by Isbell, on the rapid oxidation of aldoses to aldonic acids by hypobromite. The results showed that there was an immediate and practically quantitative formation of the δ -lactone, and good proof was given that the free aldonic acid was not an intermediate. This procedure was also applicable to the available α - and β -forms of the aldoses, and significant differences in the rates of oxidation of the α - and β -isomers were noted. The data indicate that the ordinary forms of the sugars possess pyranose structures.



A very interesting result obtained by Isbell¹²² (1933) was that a calcium chloride compound of mannose, isolated by Dale¹²³ (1929), which showed a peculiar and very rapid initial rotatory change in solution, produced a γ -lactone on hypobromite oxidation. Apparently this calcium chloride compound of mannose then possesses a furanose ring structure. All the above work on hypobromite oxidation rests on the premise that the unstable sugar lactones possess a δ -structure and is uncertain to the extent that this premise is uncertain.

Acyclic Sugar Structures. It has been seen how the original aldehyde formula for *d*-glucose gave way to the lactol or cyclic hemiacetal

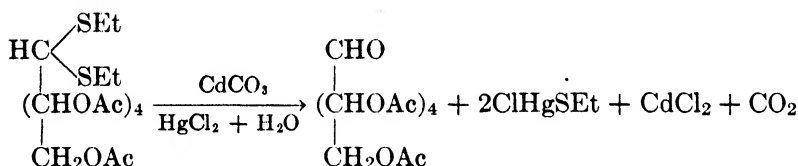
¹²¹ Isbell and Hudson, *Bur. Standards J. Research*, **8**, 327 (1932).

¹²² Isbell, *J. Am. Chem. Soc.*, **55**, 2166 (1933).

¹²³ Dale, *ibid.*, **51**, 2788 (1929).

structure required to explain further isomerism. It became a matter of considerable interest, then, when in 1926 Levene and Meyer¹²⁴ obtained a pentamethylglucose which contained no ring in its structure. This was synthesized from glucose ethyl mercaptal. Although Fischer was unable to prepare an acetal of glucose, he succeeded in preparing a thioacetal or mercaptal by reacting glucose with ethyl mercaptan in concentrated hydrochloric acid solution¹²⁵ (1894). Levene and Meyer methylated this crystalline substance and removed the thioacetal groups with mercuric chloride and water, obtaining the product as a syrup. This reaction was extended to galactose and mannose¹²⁶ (1927).

In 1929 Wolfrom¹²⁷ obtained a crystalline open chain or *aldehydo*-pentaacetate of *d*-glucose similar in structure to the above. The method used was a hydrolysis of the acetylated glucose ethyl mercaptal in dilute acetone solution by reaction with mercuric chloride in the presence of cadmium carbonate.



The substance readily formed a semicarbazone without loss of an acetate group and gave a Schiff aldehyde test. The reaction was later extended to several other sugar structures. The galactose pentaacetate¹²⁸ (1930) added a fifth crystalline pentaacetate to the four previously known. This latter substance also formed crystalline carbonyl addition compounds with alcohols and water which by their distinctive rotations were shown to be true valence compounds. In 1930 Brigl and Mühlischlegel¹²⁹ obtained an *aldehydo*-pentabenzoate of glucose which crystallized as an alcohol addition compound, apparently an ethyl hemiacetal or carbonyl addition compound. This would indicate that the nature of the substituent groups apparently influences the stability of the carbonyl group, as such addition compounds did not form with the acetate, although the mutarotation exhibited by the acetate in alcohol showed the formation of such structures in solution. The mutarotation exhibited by an *aldehydo*-acetate in alcohol can be

¹²⁴ Levene and Meyer, *J. Biol. Chem.*, **69**, 175 (1926).

¹²⁵ Fischer, *Ber.*, **27**, 673 (1894).

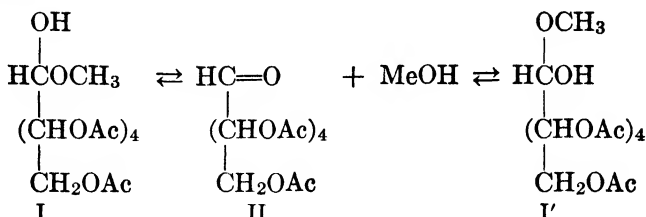
¹²⁶ Levene and Meyer, *J. Biol. Chem.*, **74**, 695 (1927).

¹²⁷ Wolfrom, *J. Am. Chem. Soc.*, **51**, 2188 (1929).

¹²⁸ Wolfrom, *ibid.*, **52**, 2464 (1930).

¹²⁹ Brigl and Mühlischlegel, *Ber.*, **63**, 1551 (1930).

explained by carbon one becoming asymmetric through hemiacetal formation.



A typical sugar mutarotation curve is obtained when an *aldehydo*-acetate (II) is dissolved in methanol, and a similar type was found by Wolfrom and Morgan¹³⁰ (1932) for *aldehydo*-galactose pentaacetate methyl hemiacetal (I) in methanol solution. The three-membered nature of the *aldehydo*-acetate and methanol equilibrium can be demonstrated by the complex nature of the mutarotation curve obtained when I is dissolved in pure chloroform. The free carbonyl form (II) of an *aldehydo*-acetate shows no mutarotation in pure chloroform. Galactose pentaacetate aldehydrol exhibits no mutarotation in water, carbon one not being asymmetric; but in chloroform solution the water dissociates from the carbonyl and a monomolecular decomposition curve results.

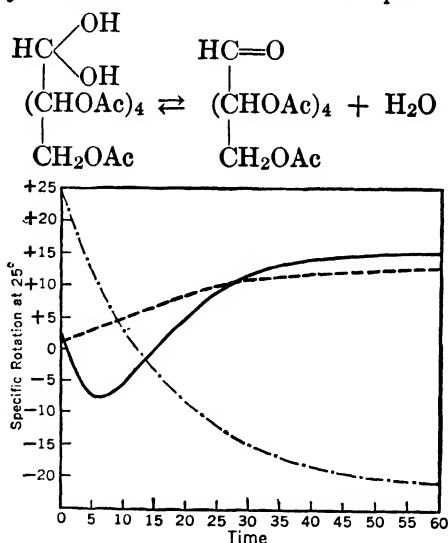


FIG. 3.—Mutarotation characteristics of *aldehydo*-sugar acetates. —, *aldehydo*-d-galactose methyl hemiacetal in chloroform (alcohol-free), time in hours; —, *aldehydo*-d-galactose methyl hemiacetal in methanol, time in hours; - · - ·, *aldehydo*-d-galactose aldehydrol in chloroform (alcohol-free), time in minutes.

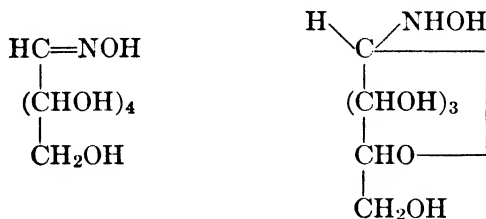
¹³⁰ Wolfrom and Morgan, *J. Am. Chem. Soc.*, **54**, 3390 (1932).

Micheel¹³¹ (1935) and Wolf from¹³² (1935) prepared fully acetylated aldose sugars in which the carbonyl group was in the diacetate form. Pirie¹³³ (1936) obtained these structures by the acetylation of sugar mercaptals with acetic anhydride and sulfuric acid and isolated *d,l*-galactose heptaacetate from agar by the use of these reagents. An acyclic acetohalogen galactose¹³⁴ has been reported, and the similar structure in the arabinose series has been found by Felton and W. Freudenberg¹³⁵ (1935) as a by-product in the preparation of the cyclic form of acetobromoarabinose (p. 1455).



A striking result was obtained by Pacsu and Rich¹³⁶ (1932), when they demonstrated that an open-chain or *keto*-structure was present in a pentaacetate of fructose prepared long before by Hudson and Brauns¹³⁷ (1915) by direct acetylation methods. A similar finding was made by Montgomery and Hudson¹³⁸ (1934), when they discovered an *aldehydo*-structure in a hexaacetate of *d*-[α -mannoheptose] obtained by acetylation of the free sugar. *Keto*-fructose pentaacetate exhibits the properties of a hindered ketone, the carbonyl group being very unreactive.

Solutions of the lactol forms of the reducing sugars show the presence of their potential carbonyl group by forming typical amino condensation products, such as phenylhydrazones, oximes, and semicarbazones. For these, however, two types of structure are theoretically possible.



¹³¹ Micheel, Ruhkopf, and Suckfüll. *Ber.*, **68**, 1523 (1935).

¹³² Wolf from, *J. Am. Chem. Soc.*, **57**, 2498 (1935).

¹³³ Pirie, *Biochem. J.*, **30**, 374 (1936).

¹³⁴ Wolf from, *J. Am. Chem. Soc.*, **57**, 2498 (1935).

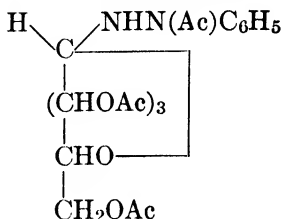
¹³⁵ Felton and W. Freudenberg, *ibid.*, **57**, 1637 (1935).

¹³⁶ Pacsu and Rich, *ibid.*, **54**, 1697 (1932).

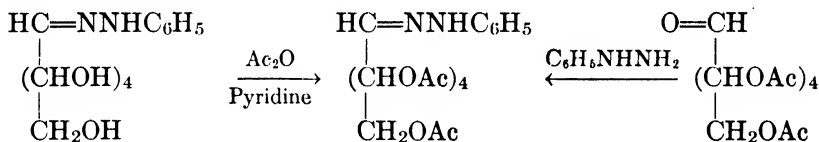
¹³⁷ Hudson and Brauns, *ibid.*, **37**, 1283 (1915).

¹³⁸ Montgomery and Hudson, *ibid.*, **56**, 2463 (1934).

When a sugar amino condensation product is acetylated, the acetate obtained may be of either type, and frequently a mixture of both is produced. Glucose phenylhydrazone exists in two forms, one of which was shown by Behrend and Reinsberg¹³⁹ (1910) to produce on acetylation a pentaacetate of the ring-structure type.



Behrend isolated α -acetylphenylhydrazine on hydrolysis of this acetate. He thus allocated one of the five acetate groups in the acetylated hydrazone to the nitrogen and furnished excellent proof for a ring structure. Had the structure been acyclic, a hexaacetate would have been indicated. This principle of establishing ring structures by detecting the presence of an N-acetyl group can be readily effected by analytical methods that distinguish between N-acetyl and O-acetyl.¹⁴⁰ The same result can be obtained by comparing the acetylated nitrogen compound with that obtained directly by reaction of the *aldehydo*-acetate with the amino reagent.



By these methods the structures of a number of acetylated sugar amino condensation products have been demonstrated.^{141, 142} Thus, glucose (and galactose) phenylosazone tetraacetate is acyclic; galactose phenylhydrazone pentaacetate is acyclic; and both the acyclic (*aldehydo*) and ring types of acetylated oximes and semicarbazones have been found. When the acetylated oxime or semicarbazone is acyclic, the *aldehydo*-form of the sugar acetate may be obtained by treatment with nitrous acid.¹⁴³

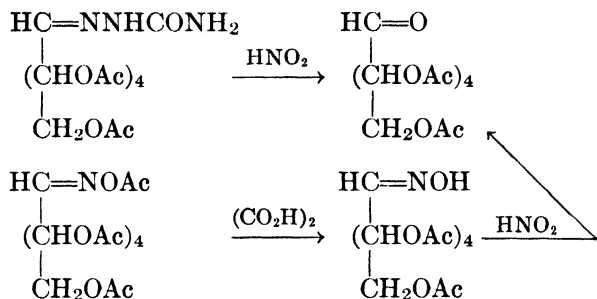
¹³⁹ Behrend and Reinsberg, *Ann.*, **377**, 189 (1910).

¹⁴⁰ Wolfrom, Konigsberg, and Soltzberg, *J. Am. Chem. Soc.*, **58**, 490 (1936).

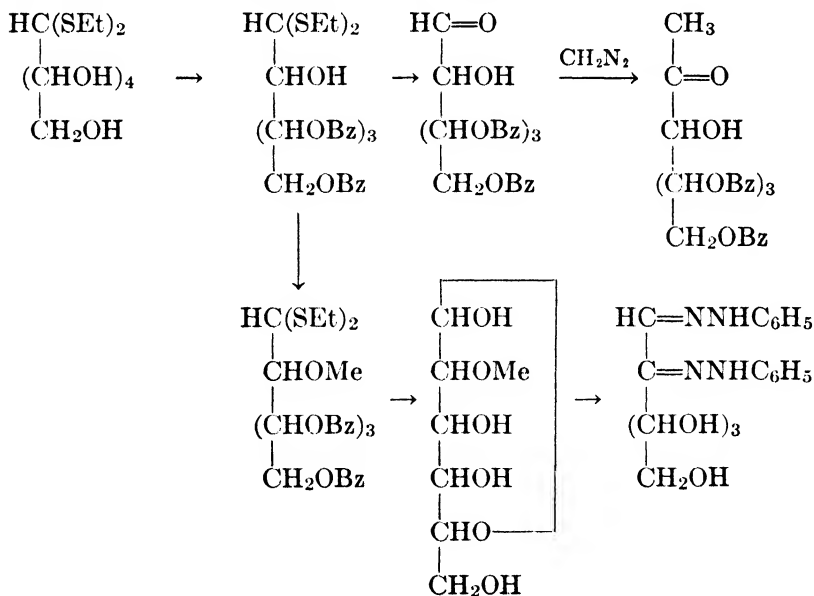
¹⁴¹ Wolfrom and Thompson, *ibid.*, **53**, 622 (1931); Wolfrom and Christman, *ibid.*, **53**, 3413 (1931); Wolfrom, Georges, and Soltzberg, *ibid.*, **56**, 1794 (1934); **58**, 1781, 1783 (1936).

¹⁴² Deulofeu, Wolfrom, Cattaneo, Christman, and Georges, *ibid.*, **55**, 3488 (1933); Deulofeu, Cattaneo, and Mendivelzua, *J. Chem. Soc.*, 147 (1934).

¹⁴³ Wolfrom and Georges, *J. Am. Chem. Soc.*, **56**, 1794 (1934).



Other Ring Structures. It is of great interest to note that Brigl and co-workers¹⁴⁴ (1931) obtained a tetrabenzoate of glucose with the second position open. This very interesting substance showed no tendency to form a lactol or ethylene oxide ring, but showed all the properties of a true open-chain α -hydroxy aldehyde, reacting with diazomethane to form a methyl ketone. This eliminates the ethylene oxide ring as a possibility with glucose, at least in its benzoate structure. The reactions employed by Brigl are diagrammed below.



Micheel and co-workers¹⁴⁵ (1933) obtained a galactose tetraacetate with the sixth position open, and this on further acetylation yielded two

¹⁴⁴ Brigl, Mühlischlegel, and Schinle, *Ber.*, **64**, 2921 (1931).

¹⁴⁵ Micheel and Suckfüll, *Ann.*, **502**, 85 (1933); **507**, 133 (1933); *Ber.*, **66**, 1957 (1933); Micheel and Spruck, *Ber.*, **67**, 1665 (1934).

new pentaacetates of galactose. The pentaacetate was transformed to a new methylgalactoside (β -methyl-*d*-galactoheptanoside) through the acetochloro compound, and this was proved to have a (1,6-) ring by methylation and oxidation. The methylheptanoside had the same low stability toward acid hydrolysis as the methylfuranosides. The reactions employed by Micheel are diagrammed below. They include several reactions of wide application in the sugar series which will be described in more detail in succeeding pages.

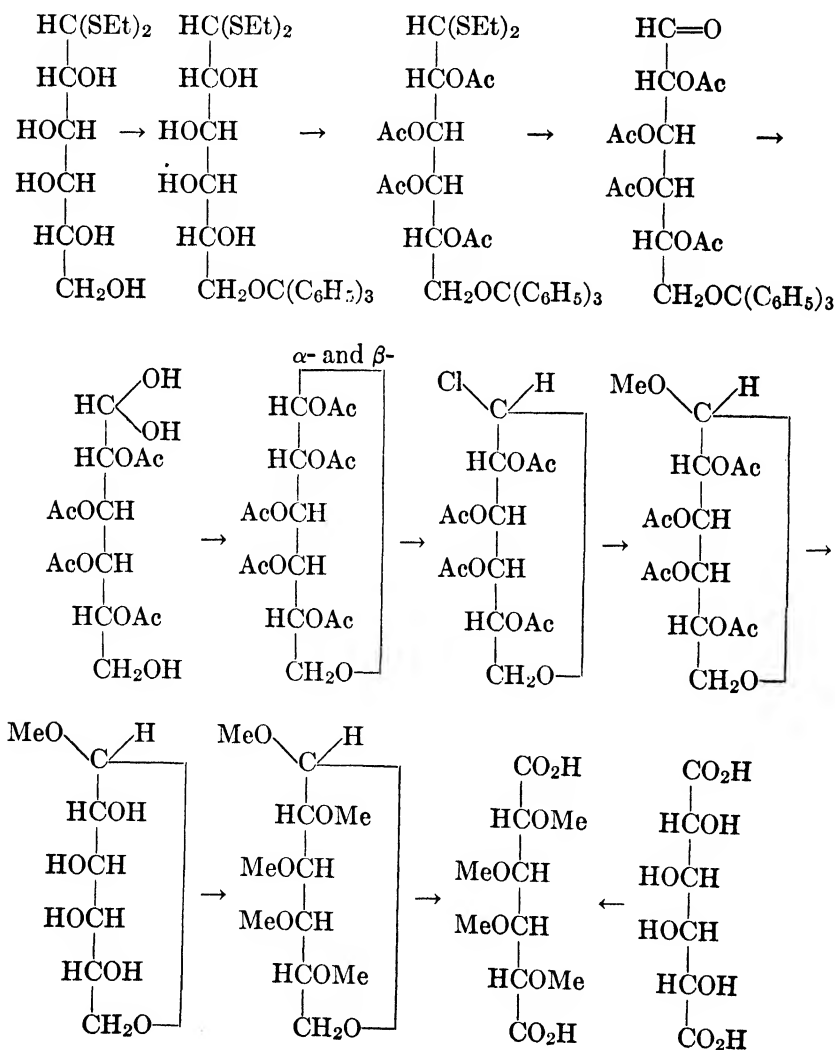
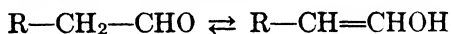


TABLE III
ISOMERIC PENTAACETATES OF *d*-GALACTOSE

Pentaacetate of:	M.P.	$[\alpha]_D$ Chloroform	2A
α - <i>d</i> -Galactopyranose	96°	+107°	+32,700
β - <i>d</i> -Galactopyranose	142	+ 23	
α - <i>d</i> -Galactofuranose (?)	87	+ 61	+40,200
β - <i>d</i> -Galactofuranose (?)	98	- 42	
α - <i>d</i> -Galactoheptanose	128	- 11	+35,900
β - <i>d</i> -Galactoheptanose	112	-103	
Aldehydo- <i>d</i> -galactose	121	- 25	

Enolic Structure. One other phase of the glucose structure requires attention and that is the enolic form. As the general chemistry of the carbonyl group has developed, it has become evident that one of its main reactions is that of enolization.



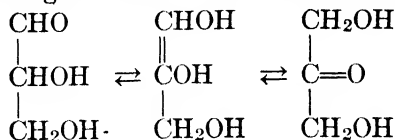
The extent of such spontaneous enolization varies considerably with the nature of the carbonyl compound, but is greatly enhanced by alkalinity. There is also good evidence that the enolic form is the intermediate in various reactions, the aldehyde or ketone shifting over to this form as the enol is consumed in the reaction. It would be strange indeed if the aldehyde glucose were an exception to these well-established principles. The indirect evidence for the existence of the enolic sugar structures is impressive and is to be found especially in the complicated reactions that take place when the reducing sugars are placed in alkaline media. The distinctive peculiarity of the sugar enolic form is that this is not an ordinary enol, but an enediol. The acyclic or *aldehydo* form is required as an intermediate for enediol formation. Marchlewski¹⁴⁶ (1933) has shown that a carbonyl absorption band appears immediately on the addition of alkali to glucose and other reducing sugars and disappears on neutralization of the alkali. It is also well known that a slight alkalinity is essential for the cyanohydrin reaction, in which the acyclic form of the sugar is the logical intermediate. Supporting evidence for such an intermediate is furnished by measurements of the initial hydrogen cyanide binding capacity of sugars^{147, 148} (Lippich, 1932; Brigl, 1931).

¹⁴⁶ Gabryelski and Marchlewski, *Biochem. Z.*, **261**, 393 (1933).

¹⁴⁷ Lippich, *ibid.*, **248**, 280 (1932).

¹⁴⁸ Brigl, Mühlischlegel, and Schinle, *Ber.*, **64**, 2921 (1931).

In 1900 Wohl and Neuberg¹⁴⁹ observed that glyceraldehyde, when heated in an aqueous pyridine solution, was converted into an equal mixture of dihydroxyacetone and glyceraldehyde. They explained this reaction by postulating an intermediate enolic structure.



This explanation was adopted by Nef to interpret the experiments of Lobry de Bruyn and Alberda van Ekenstein¹⁵⁰ (1896). These last-named workers showed that the sugars mannose and fructose appeared when glucose was placed in dilute alkaline solution. The detailed consideration of this reaction will be reserved for the succeeding chapter (p. 1509).

The Lobry de Bruyn dilute alkali interconversion reaction was used successfully by Montgomery and Hudson¹⁵¹ (1930) in obtaining the crystalline ketose of lactose (lactulose) and by Austin¹⁵² (1930) for *d*-glucoheptulose. At this point it would be well to mention the only other successful method for ketose synthesis at present available. This is the biological method developed by Bertrand. In 1852, the French scientist, Pelouze,¹⁵³ described the isolation of a new ketohexose from the juice of the berries of the mountain ash. This was a readily crystallizable sugar which has been named sorbose. For half a century, attempts to repeat the experiments of Pelouze remained unsuccessful, until Bertrand¹⁵⁴ (1896) found that the mountain ash synthesized merely the alcohol *d*-sorbitol and that this was oxidized to the ketose by a bacterium introduced into the fruit by a type of vinegar fly. Bertrand isolated this bacterium, now known as the sorbose bacterium (*Acetobacter xylinum*), and with this in hand it was a rather simple problem for him to prepare the ketose from pure *d*-sorbitol. The ketose so produced is *l*-sorbose, and a transformation from the *d*- to the *l*-sugar series is thus effected.

This method for ketose synthesis, being biological, is strictly limited in application to those alcohols having a *cis* configuration on carbon atoms two and three or four and five. Bertrand¹⁵⁵ (1904) made crystalline dihydroxyacetone available by the action of the bacterium

¹⁴⁹ Wohl and Neuberg, *Ber.*, **33**, 3095 (1900).

¹⁵⁰ Lobry de Bruyn and Alberda van Ekenstein, *Rec. trav. chim.*, **15**, 93 (1896).

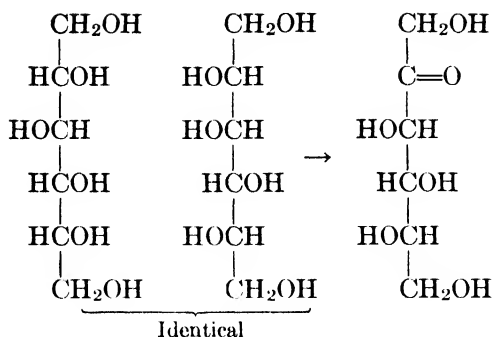
¹⁵¹ Montgomery and Hudson, *J. Am. Chem. Soc.*, **52**, 2101 (1930).

¹⁵² Austin, *ibid.*, **52**, 2106 (1930).

¹⁵³ Pelouze, *Ann. chim. phys.*, [3] **35**, 222 (1852).

¹⁵⁴ Bertrand, *Bull. soc. chim.*, [3] **15**, 627 (1896).

¹⁵⁵ Bertrand, *Ann. chim. phys.*, [8] **3**, 230 (1904).



on glycerol. He also obtained crystalline *l*-glucoheptulose¹⁵⁶ (1928), and a crystalline ketoheptose, perseulose¹⁵⁷ (1909), from perseitol, a naturally occurring heptitol. The known ketose sugars are tabulated in Table IV.

TABLE IV
KETOSE SUGARS

Name	Structure	Occurrence
Dihydroxyacetone	$\text{CH}_2\text{OHCOCH}_2\text{OH}$	
<i>l</i> -Erythrulose (syrup)	$ \begin{array}{c} \text{CH}_2\text{OH}-\text{C}-\text{COCH}_2\text{OH} \\ \\ \text{H} \\ \\ \text{H} \quad \text{OH} \end{array} $	
<i>d</i> -Xyloketose (syrup)	$ \begin{array}{c} \text{CH}_2\text{OH}-\text{C}-\text{C}-\text{COCH}_2\text{OH} \\ \quad \\ \text{OH} \quad \text{H} \\ \quad \\ \text{OH} \quad \text{H} \end{array} $	
<i>l</i> -Xyloketose (syrup)	$ \begin{array}{c} \text{CH}_2\text{OH}-\text{C}-\text{C}-\text{COCH}_2\text{OH} \\ \quad \\ \text{H} \quad \text{OH} \\ \quad \\ \text{H} \quad \text{H} \end{array} $	Pentosuric urine
<i>d</i> -Riboketose (syrup)	$ \begin{array}{c} \text{CH}_2\text{OH}-\text{C}-\text{C}-\text{COCH}_2\text{OH} \\ \quad \\ \text{OH} \quad \text{OH} \\ \quad \\ \text{OH} \quad \text{OH} \end{array} $	
<i>l</i> -Riboketose (syrup)	$ \begin{array}{c} \text{CH}_2\text{OH}-\text{C}-\text{C}-\text{COCH}_2\text{OH} \\ \quad \\ \text{H} \quad \text{H} \end{array} $	
β - <i>d</i> -Fructose	$ \begin{array}{c} \text{OCH}_2-\text{C}-\text{C}-\text{C}-\text{C}-\text{CH}_2\text{OH} \\ \quad \quad \quad \\ \text{H} \quad \text{H} \quad \text{OH} \quad \text{OH} \\ \quad \quad \quad \\ \text{OH} \quad \text{OH} \quad \text{H} \quad \text{H} \end{array} $	Fruits; honey

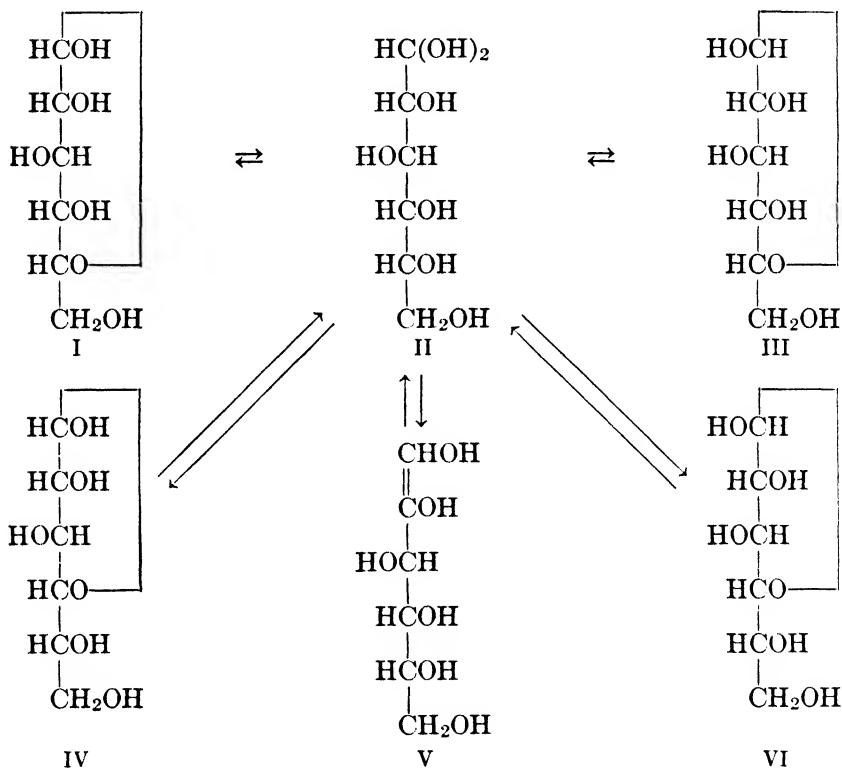
¹⁵⁶ Bertrand and Nitzberg, *Compt. rend.*, **186**, 1172 (1928).

¹⁵⁷ Bertrand, *Bull. soc. chim.*, [4] **5**, 629 (1909).

TABLE IV—Continued
KETOSE SUGARS

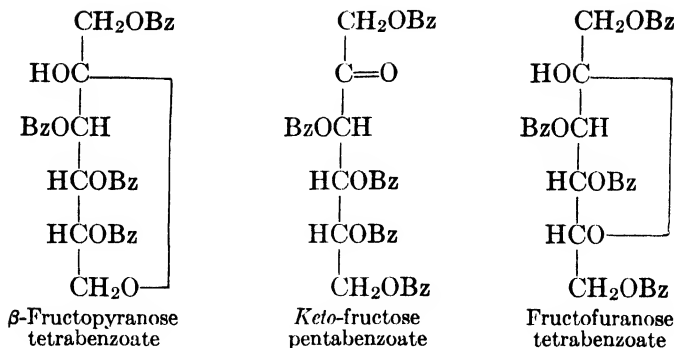
Name	Structure	Occurrence
<i>l</i> -Fructose (syrup)	$ \begin{array}{ccccccc} & \text{OH} & \text{OH} & \text{H} & & & \\ & & & & & & \\ \text{CH}_2\text{OH} & -\text{C}- & \text{C}- & \text{C}- & \text{COCH}_2\text{OH} \\ & & & & & & \\ & \text{H} & \text{H} & \text{OH} & & & \\ & & & & & & \\ & \text{H} & \text{OH} & \text{H} & & & \\ \text{CH}_2\text{OH} & -\text{C}- & \text{C}- & \text{C}- & \text{COCH}_2\text{OH} \\ & & & & & & \\ & \text{OH} & \text{H} & \text{OH} & & & \\ & & & & & & \\ & \text{OH} & \text{H} & \text{OH} & & & \\ \text{CH}_2\text{OH} & -\text{C}- & \text{C}- & \text{C}- & \text{COCH}_2\text{OH} \\ & & & & & & \\ & \text{H} & \text{OH} & \text{H} & & & \\ & & & & & & \\ & \text{H} & \text{OH} & \text{OH} & & & \\ \text{CH}_2\text{OH} & -\text{C}- & \text{C}- & \text{C}- & \text{COCH}_2\text{OH} \\ & & & & & & \\ & \text{OH} & \text{H} & \text{H} & & & \\ & & & & & & \\ & \text{OH} & \text{OH} & \text{OH} & & & \\ \text{CH}_2\text{OH} & -\text{C}- & \text{C}- & \text{C}- & \text{COCH}_2\text{OH} \\ & & & & & & \\ & \text{H} & \text{H} & \text{H} & & & \\ & & & & & & \\ & \text{H} & \text{H} & \text{H} & \text{OH} & & \\ \text{CH}_2\text{OH} & -\text{C}- & \text{C}- & \text{C}- & \text{C}- & \text{COCH}_2\text{OH} \\ & & & & & & \\ & \text{OH} & \text{OH} & \text{OH} & \text{H} & & \\ & & & & & & \\ & \text{H} & \text{H} & \text{OH} & \text{OH} & & \\ \text{CH}_2\text{OH} & -\text{C}- & \text{C}- & \text{C}- & \text{C}- & \text{COCH}_2\text{OH} \\ & & & & & & \\ & \text{OH} & \text{OH} & \text{H} & \text{H} & & \\ & & & & & & \\ & \text{OH} & \text{H} & \text{H} & \text{OH} & & \\ \text{CH}_2\text{OH} & -\text{C}- & \text{C}- & \text{C}- & \text{C}- & \text{COCH}_2\text{OH} \\ & & & & & & \\ & \text{H} & \text{OH} & \text{OH} & \text{H} & & \\ & & & & & & \\ & \text{H} & \text{H} & \text{OH} & \text{H} & & \\ \text{CH}_2\text{OH} & -\text{C}- & \text{C}- & \text{C}- & \text{C}- & \text{COCH}_2\text{OH} \\ & & & & & & \\ & \text{OH} & \text{OH} & \text{H} & \text{OH} & & \\ & & & & & & \\ & \text{OH} & \text{OH} & \text{H} & \text{OH} & & \\ \text{CH}_2\text{OH} & -\text{C}- & \text{C}- & \text{C}- & \text{C}- & \text{COCH}_2\text{OH} \\ & & & & & & \\ & \text{H} & \text{H} & \text{OH} & \text{H} & & \end{array} $	
<i>d</i> -Sorbose		
<i>l</i> -Sorbose		
<i>d</i> -Tagatose		
<i>l</i> -Psicose (syrup)		
Sedoheptose (syrup)		<i>Sedum spectabile</i>
Mannoketoheptose		Avocado pear
Perseulose		
<i>d</i> -Glucoheptulose		
<i>l</i> -Glucoheptulose		
Turanose	Disaccharide; hydrolytic product of melezitose	
Lactulose	Ketose of the disaccharide lactose	

Summary of Ring Structure and Tautomeric Forms. All the preceding evidence shows definitely that glucose is a highly tautomeric substance. To be sure, the two known crystalline forms of this sugar undoubtedly have the pyranose structure, but when these are brought into solution many changes may occur. Evidence has been obtained for all the following glucose structures. The stable or resting stages are the pyranose forms, I and III; IV and VI are perhaps favored by acidity and II and V by alkalinity.



Brigl and Schinle¹⁵⁸ (1933; 1934) have obtained direct evidence for the high degree of tautomerism displayed by fructose. The marked changes in rotation with temperature exhibited by aqueous solutions of fructose indicated that this sugar was highly tautomeric. Brigl and Schinle isolated three crystalline benzoates by the direct benzylation of fructose and furnished good evidence that they were, respectively:

¹⁵⁸ Brigl and Schinle, *Ber.*, **66**, 325 (1933); **67**, 127 (1934).



The ring structure of the tetroses is of interest. These sugars cannot exist in the monomolecular form as a pyranose ring, but only in a furanose or smaller ring. None of the tetrose sugars has so far been obtained crystalline. The molecular weight of an erythrose syrup has been found to be that of a normal monomer by Deulofeu¹⁵⁹ (1932). This might have been expected from the previous work of Helferich and his collaborators¹⁶⁰ (1921) with γ -hydroxy aldehydes. An outstanding advance in tetrose chemistry has been the isolation of a crystalline diacetate of *d*-threose by W. Freudenberg¹⁶¹ (1932) and of a crystalline triacetate of the same by Hockett¹⁶² (1934). Swan and Evans¹⁶³ (1935) have also obtained the first crystalline glycoside of a tetrose (α -methyl-*l*-arabomethylloside).

The present rather considerable knowledge of the trioses glyceraldehyde and dihydroxyacetone and of the one diose, glycolaldehyde, is due in large part to the careful work of H. O. L. Fischer and his students in this very difficult field. The crystalline forms of glycolaldehyde and of glyceraldehyde are dimeric, but molecular-weight determinations show that they pass spontaneously into the monomer in aqueous solution. The derivatives of these aldoses are likewise dimeric. It is probable that the dimeric structures are produced by the carbonyl of one molecule forming a lactol with the hydroxyl hydrogen of a second molecule to produce a six-membered ring of the dioxane type.

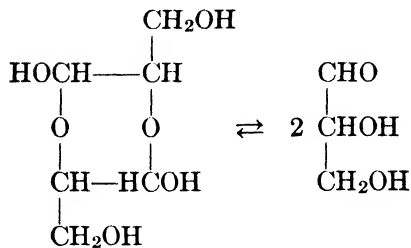
¹⁵⁹ Deulofeu, *J. Chem. Soc.*, 2973 (1932).

¹⁶⁰ Helferich and Lecher, *Ber.*, **54**, 930 (1921).

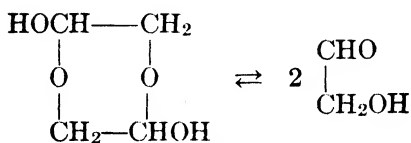
¹⁶¹ W. Freudenberg, *Ber.*, **65**, 168 (1932).

¹⁶² Hockett, *J. Am. Chem. Soc.*, **56**, 994 (1934).

¹⁶³ Swan and Evans, *ibid.*, **57**, 200 (1935).



Glyceraldehyde



Glycolaldehyde

OXIDIZED SUGAR STRUCTURES

In addition to the mono- and dibasic sugar acids previously discussed, a number of other oxidized sugar structures are of interest.

Osones. The term osone, used to denote the sugar dicarbonyl compounds, was originally introduced by Fischer¹⁶⁴ (1889) to name the syrupy product obtained by the hydrolysis of a phenylosazone with hydrochloric acid¹⁶⁵ (1888). Glucosone (1,2-) has the general structure $\text{CH}_2\text{OH}-(\text{CHOH})_3-\text{CO}-\text{CHO}$. Its ring structure has not been clarified. A crystalline tetraacetate monohydrate of this substance has been obtained by Maurer¹⁶⁶ (1929). Glucosone is an intermediate in the oxidation of glucose with copper acetate and in the Ruff oxidative degradation. Hexosones having the second carbonyl group in other positions than the second carbon have been obtained by the general synthetic reactions established by Maurer.

Keto Acids. An interesting oxidation product of glucose is the ketogluconic acid obtained long ago and reinvestigated by Kiliani¹⁶⁷ (1922; 1925). The keto group is on the fifth carbon in this keto acid. The 2-keto acids of the sugar group are of especial importance and will be discussed in the succeeding chapter (p. 1506).

Glycuronic Acids. The pioneer physiological chemist Schmiedeberg¹⁶⁸ (1879) found that when camphor was fed to an animal it was eliminated in the urine as a bornylglycoside in which the terminal pri-

¹⁶⁴ Fischer, *Ber.*, **22**, 88 (1889).

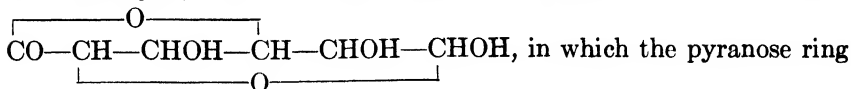
¹⁶⁵ Fischer, *Ber.*, **21**, 2632 (1888).

¹⁶⁶ Maurer, *Ber.*, **62**, 332 (1929).

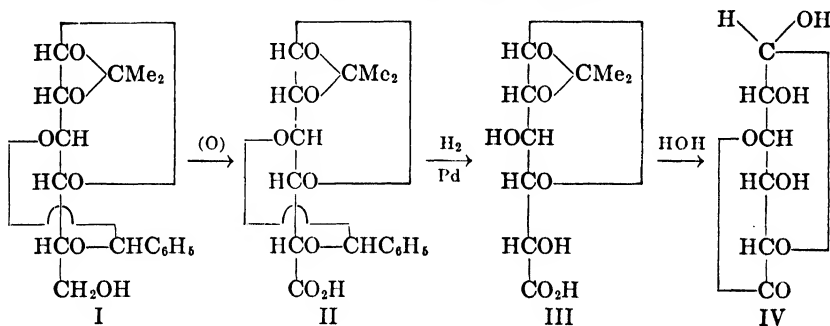
¹⁶⁷ Kiliani, *Ber.*, **55**, 75, 2817 (1922); **58**, 2344 (1925).

¹⁶⁸ Schmiedeberg and Meyer, *Z. physiol. Chem.*, **3**, 422 (1879).

mary hydroxyl group of glucose had been oxidized to the carboxyl group. On hydrolysis, this produced crystalline glucuronic acid lactone,



structure is indicated by the methylation experiments of Pryde and Williams¹⁶⁹ (1933) on bornyl glucuronate. According to Quick¹⁷⁰ (1927), glucuronic acid can be most conveniently prepared from bornyl glucuronate, obtained by administering borneol to dogs. Zervas and Sessler¹⁷¹ (1933) have synthesized glucuronic acid from acetonebenzylideneglucose (I). The free terminal primary alcohol group of I was oxidized with alkaline permanganate solution to the acid II, which on catalytic hydrogenation with palladium produced acetoneglucuronic acid (III), from which the glucuronic acid lactone (IV) was obtained by mild acid hydrolysis. Fischer and Piloty¹⁷² (1891) had also synthesized IV by the reduction of the lactone of glucosaccharic acid.



The animal organism has the power of combining substances which are toxic, or which can be oxidized only slowly, with glucuronic acid and excreting them in the urine. It was once suggested that the substances first form a glucoside with glucose, which then, since the aldehyde group is protected, is oxidized at the other end of the chain. This view can no longer be upheld since Pryde and co-workers¹⁷³ (1934) have shown that phenyl- and bornyl- β -glucosides are not converted by the dog to the corresponding glucuronates. The source of glucuronic acid is probably mucin. The place of glucuronic acid in the general scheme of detoxication mechanisms of the body has been studied by Quick¹⁷⁴ (1932).

¹⁶⁹ Pryde and Williams, *Biochem. J.*, **27**, 1197 (1933).

¹⁷⁰ Quick, *J. Biol. Chem.*, **74**, 331 (1927).

¹⁷¹ Zervas and Sessler, *Ber.*, **66**, 1326 (1933).

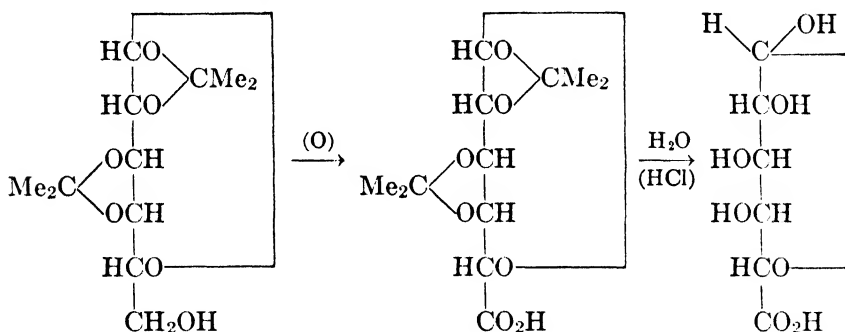
¹⁷² Fischer and Piloty, *Ber.*, **24**, 524 (1891).

¹⁷³ Hemingway, Pryde, and Williams, *Biochem. J.*, **28**, 136 (1934).

¹⁷⁴ Quick, *J. Biol. Chem.*, **97**, 403 (1932).

d-Glucuronic acid has a very widespread occurrence in the plant and animal world; with galacturonic acid, it is a constituent of the plant mucilages and gums. *d*-Glucuronic acid is found in the type specific polysaccharide of Type III pneumococcus in combination with glucose¹⁷⁵ (Heidelberger and Goebel, 1927); and a similar substance, *d*-galactopyranose-6-glucuronate, is a partial hydrolysis product of gum arabic. The extended investigations of Levene and co-workers have established the presence of glucuronic acid as a constituent of the carbohydrate portion of the mucoproteins.

d-Galacturonic acid is a component of the fruit pectins, which have been extensively investigated by Ehrlich and his students. Link¹⁷⁶ and co-workers (1931) have described its preparation in crystalline condition from technical citrus pectin. Anderson¹⁷⁷ has made the interesting observation that both *l*-galactose and *d*-galacturonic acid are hydrolytic products of flaxseed mucilage. Both the *d*- and the *l*-forms of galacturonic acid have been synthesized from diacetone-(1,2; 3,4)-galactose by Niemann and Link¹⁷⁸ (1934).



Two crystalline forms of *d*-galacturonic acid are known, and to one of them Ehrlich assigns the aldehyde-hydrate formula. Two crystalline methylgalacturonides have also been prepared (Morell and Link,¹⁷⁹ 1932; Ehrlich and Guttman,¹⁸⁰ 1933), and evidence based upon kinetics of hydrolysis is offered for a pyranoside ring in the α -isomer.

The lactone of *d*-mannuronic acid has been isolated from certain types of seaweed by Nelson and Cretcher¹⁸¹ (1930). This lactone has

¹⁷⁵ Heidelberger and Goebel, *ibid.*, **74**, 613 (1927).

¹⁷⁶ Link and Neddén, *ibid.*, **94**, 307 (1931); Morell, Baur, and Link, *ibid.*, **105**, 15 (1934).

¹⁷⁷ Anderson, *ibid.*, **100**, 249 (1933).

¹⁷⁸ Niemann and Link, *ibid.*, **104**, 195, 743 (1934).

¹⁷⁹ Link, *Nature*, **130**, 402 (1932); Morell and Link, *J. Biol. Chem.*, **100**, 385 (1933).

¹⁸⁰ Ehrlich and Guttman, *Ber.*, **66**, 220 (1933).

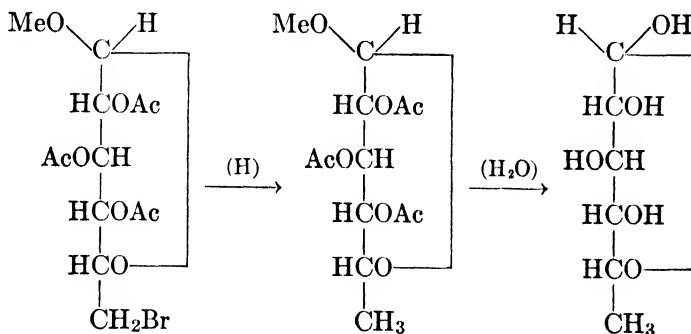
¹⁸¹ Nelson and Cretcher, *J. Am. Chem. Soc.*, **52**, 2130 (1930).

also been synthesized by the reduction of *d*-mannosaccharic acid lactone (Niemann and Link,¹⁸² 1933); similar synthetic procedures have yielded *l*-mannuronolactone and *d,l*-alluronic acid (Link and co-workers, 1934¹⁸³). The ready decarboxylation of uronic acids forms the basis of a widely used analytical method for their estimation, originally devised by Lefèvre and Tollens¹⁸⁴ (1907).

REDUCED SUGAR STRUCTURES

In addition to the previously described sugar alcohols, a number of sugar reduction products are known in which one of the hydroxyl groups of the parent sugar has been replaced by hydrogen.

Methylpentoses. A group of naturally occurring sugars are known which may be considered as hexose reduction products, in which the terminal primary hydroxyl group of the hexose has been reduced to a methyl group (p. 1502). They are commonly termed the methylpentoses. On treatment with mineral acids they decompose to form methylfurfuraldehyde; under these conditions the pentoses yield furfuraldehyde. There are as many possible stereoisomeric methylpentoses (6-desoxyhexoses) as there are hexoses, and Votoček has simplified their nomenclature by using the general suffix *-methyllose*. *d*-Glucomethyllose, a crystalline sugar, is a constituent of the glycoside convolvulin. Its structure was determined by Fischer and Zach¹⁸⁵ (1912) beyond doubt by its synthesis from the methylglycoside of 6-bromo-*d*-glucose triacetate through reduction of the bromine atom by means of zinc dust and acetic acid. The triacetyl derivative obtained yielded a glycoside on alkaline hydrolysis from which the methylpentose was finally obtained on acid hydrolysis.



¹⁸² Niemann and Link, *J. Biol. Chem.*, **100**, 407 (1933).

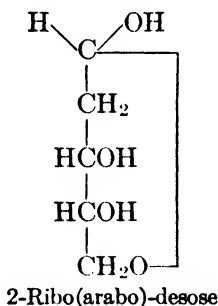
¹⁸³ Niemann, McCubbin, and Link, *ibid.*, **104**, 737 (1934); Niemann, Karjala, and Link, *ibid.*, **104**, 189 (1934).

¹⁸⁴ Lefèvre and Tollens, *Ber.*, **40**, 4517 (1907).

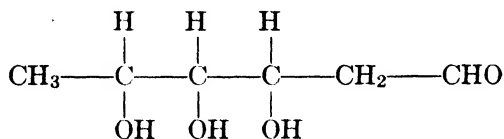
¹⁸⁵ Fischer and Zach, *Ber.*, **45**, 3761 (1912).

The best-known methylpentose is rhamnose or *l*-mannomethylose. This well-crystallized sugar is a constituent of many glycosides, the best known of which is quercitrin, the glycoside of oak bark. *l*-Fucose or *l*-galactomethylose is obtained by the acid hydrolysis of certain types of seaweed, and its enantiomorph, *d*-fucose or rhodose, is a constituent of the glycoside jalapin. The present knowledge of the occurrence and structure of these reduced hexoses is due in large part to the work of Votoček and his students.

2-Desoxy Sugars. Another group of reduced sugars are the 2-desoxy sugars or 2-desoses in which carbon two of a hexose or pentose has been reduced to a methylene group. 2-Glucodesose was prepared synthetically by Bergmann and co-workers¹⁸⁶ (1922) through a series of reactions involving the reduction of acetobromoglucose, the details of which are left for the next chapter (p. 1501). Considerable interest has been attached to desoxy sugars by the isolation of crystalline 2-ribodesose (2-desoxyribose) as the sugar of thymus nucleic acid by Levene and London¹⁸⁷ (1928). The glycosides of the 2-desoxy sugars are as unstable toward acid hydrolysis as the furanosides although methylation studies indicate that pyranose lactols are present.



2,6-Desoxy Sugars. The digitalis group (p. 1345) of glycosides produce on hydrolysis a number of interesting sugars that were first investigated by Kiliani. From digitoxin, Kiliani¹⁸⁸ (1895) isolated a sugar which he named digitoxose and to which the following formula is now assigned:

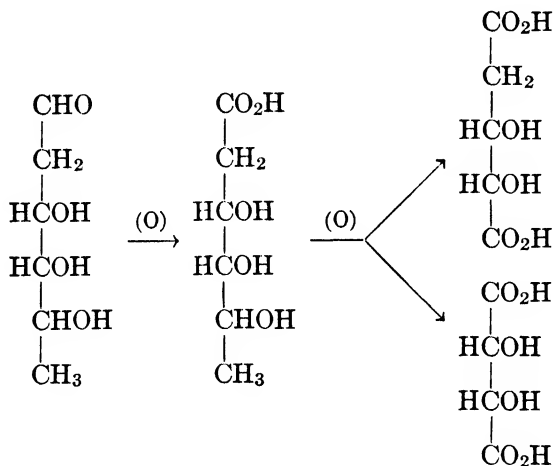


¹⁸⁶ Bergmann, Schotte, and Lechinsky, *Ber.*, **55**, 158 (1922); **56**, 1052 (1923).

¹⁸⁷ Levene and London, *J. Biol. Chem.*, **81**, 711 (1928); **83**, 793 (1929).

¹⁸⁸ Kiliani, *Arch. Pharm.*, **233**, 319 (1895).

Kiliani established the formula $C_6H_{12}O_4$ for this sugar; the formula thus differed from that of an ordinary hexose ($C_6H_{12}O_6$) by having a deficiency of two oxygen atoms. He noted that the sugar was an aldose by the formation of its aldonic acid on hypobromite oxidation. It formed a phenylhydrazone but no osazone¹⁸⁹ (1896); thus carbon two was reduced. Acetic acid was formed on oxidation with silver oxide¹⁹⁰ (1899); therefore a terminal methyl group was present. Kiliani¹⁹¹ later (1905) obtained α,β -dihydroxyglutaric acid and meso-tartaric acid on nitric acid oxidation; this was used as proof that the hydroxyls on carbons three and four were *cis*.



Starting with a substance (II) which was obtained by Cloetta¹⁹² (1920) through vacuum sublimation of digitoxin and which was known to possess an anhydro structure of the glycal type (Windaus and Schwarte,¹⁹³ 1926), Micheel¹⁹⁴ (1930) obtained on ozonization a methyltetrose (III). This (III) was shown to possess the same configuration on carbon four and carbon five of the digitoxose chain as *d*-arabomethylose, by the preparation of identical phenylosazones from both sources. Hence, since the hydroxyl groups on carbon three and carbon four are *cis* to one another, it follows that all three hydroxyl groups are *cis* and that digitoxose belongs to the *d*-series and may be named 2-desoxy-*d*-allomethylose (I).

¹⁸⁹ Kiliani, *ibid.*, **234**, 486 (1896).

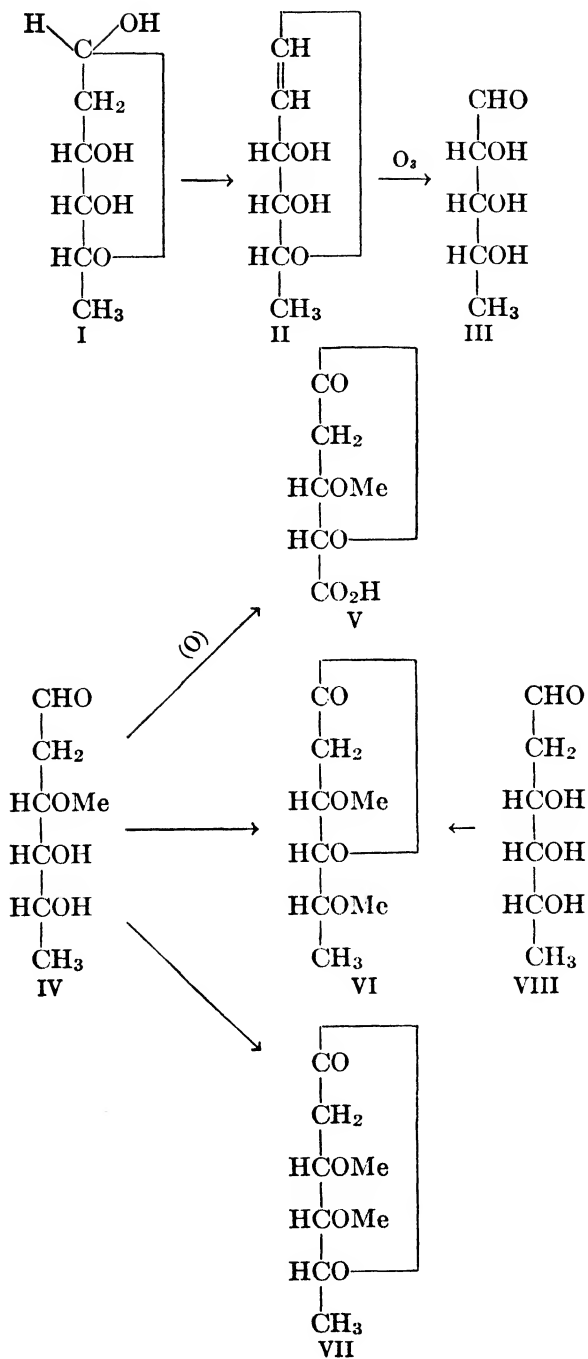
¹⁹⁰ Kiliani, *Ber.*, **32**, 2196 (1899).

¹⁹¹ Kiliani, *Ber.*, **38**, 4040 (1905).

¹⁹² Cloetta, *Arch. exp'tl. Path. Pharmacol.*, **88**, 113 (1920).

¹⁹³ Windaus and Schwarte, *Nachr. Ges. Wiss. Göttingen, Math. physik. Klasse*, **1** (1926).

¹⁹⁴ Micheel, *Ber.*, **63**, 347 (1930).

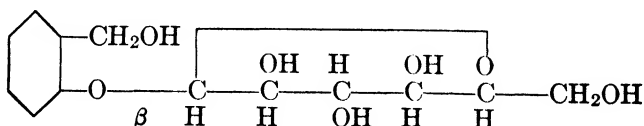


Another sugar of the digitalis group is cymarose, first obtained by Windaus and Hermanns¹⁹⁵ (1915) from cymarín. They showed it to be a methyl ether of a 2-desoxyhexomethylose and suggested that it might be a methyl ether of digitoxose. Elderfield¹⁹⁶ (1935) has shown that the methoxyl group is in position three and has demonstrated the configurational identity with digitoxose. On nitric acid oxidation, cymarose yielded the lactone of α -hydroxy- β -methoxyglutaric acid (V), thus rendering probable the allocation of the methoxyl group on carbon three. This was substantiated by the preparation of two different fully methylated lactones from cymarose, one of which possessed a γ -lactone ring (VI) and the other a δ -lactone ring (VII). Thus, both of the hydroxyl groups on carbon four and carbon five are unsubstituted. The relationship of cymarose to digitoxose (VIII) was shown by the preparation of identical fully methylated γ -lactones from both sugars. Cymarose therefore is represented by formula IV.

GLYCOSIDES

A wide variety of glycosides are found in the plant world. They are composed of alcohols or phenols in glycosidic combination with a sugar—a type of combination which has been discussed previously in dealing with the α - and β -glycopyranosides and glycofuranosides. On hydrolysis by acids or by enzymes, the glycosides produce one or more sugars, chiefly *D*-glucose, and the non-sugar portion, which is termed the aglucon. These aglucons are of a very diversified nature (pp. 1004, 1117, 1314). It is remarkable that the naturally occurring glycosides are for the most part levorotatory and are hydrolyzable by emulsin. They accordingly belong to the β -glycosides. Their function in the plant is rather obscure, but the physiological actions of many are well established, and it is to the presence of such glycosides that many herbs and roots owe their medicinal value. A few of the large number of naturally occurring glycosides are tabulated in Table V.

Salicin is a classical example of a simple glycoside. It occurs in willow bark and has the following constitution:



¹⁹⁵ Windaus and Hermanns, *Ber.*, **48**, 993 (1915).

¹⁹⁶ Elderfield, *J. Biol. Chem.*, **111**, 527 (1935).

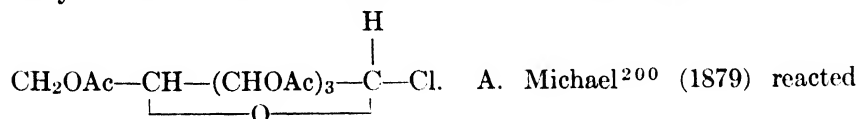
TABLE V
REPRESENTATIVE GLYCOSIDES OF NATURAL OCCURRENCE

Glycoside	Sugar	Aglucon
Populin	Benzoylglucose	<i>o</i> -Hydroxybenzyl alcohol
Coniferin	Glucose	Coniferyl alcohol
Aesculin	Glucose	6,7-Dihydroxycoumarin
Idaein	Galactose	Cyanidin (p. 1119)
Scopolin	Glucose (2 moles)	6-Methylaesculetin
Peonin	Glucose (2 moles)	Cyanidin
Violanin	Rhamnose + glucose	Delphinidin (p. 1119)
Digitoxin	Digitoxose (3 moles)	Digitoxigenin (p. 1332)
Cymarín	Cymarose	Strophanthidin (p. 1323)
Prunasin	Glucose	Mandelonitrile
Lotusin	Gentiobiose	Lotoflavin
Hesperidin	Glucose + rhamnose	Hesperitin
Salicin	Glucose	<i>o</i> -Hydroxybenzyl alcohol

Irvine and Rose¹⁹⁷ (1906) methylated this substance to form a crystalline pentamethylsalicin which produced tetramethylglucopyranose on hydrolysis. Levene and Tipson¹⁹⁸ (1931) have found from methylation studies that some of the glycosides (nucleosides) (p. 1005), produced by the partial hydrolysis of nucleic acids (p. 1011), are furanosides.

The structures of a number of the naturally occurring glycosides have been verified by synthetic methods. The previously described method of Fischer produced the isomeric glycosides directly from the sugar and alcohol. The other and more selective method is that employing the acetohalogen sugars as condensing agents.

The first acetohalogen sugar, acetochloroglucose, was prepared by Colley¹⁹⁹ (1870) by the reaction between glucose and five moles of acetyl chloride. It is now known that this substance has the structure



this compound with potassium phenolate and obtained crystalline β -phenylglucoside. In an analogous manner, Michael²⁰¹ (1881) performed the first artificial synthesis of a naturally occurring glycoside and

¹⁹⁷ Irvine and Rose, *J. Chem. Soc.*, **89**, 814 (1906).

¹⁹⁸ Levene and Tipson, *Science*, **74**, 521 (1931); *J. Biol. Chem.*, **94**, 809 (1932).

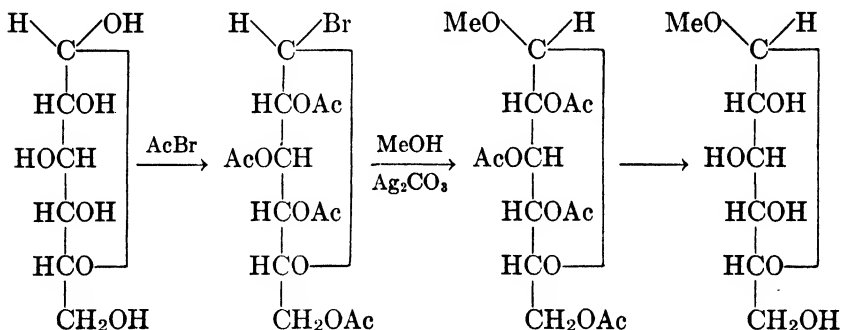
¹⁹⁹ Colley, *Ann. chim. phys.*, [4] **21**, 363 (1870).

²⁰⁰ Michael, *Am. Chem. J.*, **1**, 305 (1879).

²⁰¹ Michael, *Ber.*, **14**, 2097 (1881).

obtained methyl arbutin or $\text{CH}_3\text{O}-\text{C}_6\text{H}_{10}-\text{OC}_6\text{H}_{11}\text{O}_5$. A more reactive and useful compound than acetochloroglucose was obtained when Koenigs and Knorr²⁰² (1901) prepared crystalline acetobromoglucose by the action of acetyl bromide upon glucose. These workers showed that this compound reacted readily with methanol in the presence of silver carbonate to form β -methylglucoside tetraacetate. Koenigs and Knorr also showed that the crystalline acetoneitroglucose, obtained by Colley²⁰³ (1873), reacted in a similar manner with methanol in the presence of barium carbonate to form the tetraacetate of β -methylglucoside. Acetoneitroglucose has the structure $\text{CH}_2\text{OAcCH}-(\text{CHOAc})_3-\text{CHONO}_2$.

Deacetylation of the above glucoside tetraacetate produced the previously known β -methylglucoside.



E. Fischer²⁰⁴ (1911) improved the method for preparing the acetohalogen sugars by employing the reaction between the hexose pentaacetate (either α - or β -) and a glacial acetic acid solution of the halogen acid. In this manner, acetohalogen sugars containing chlorine, bromine, or iodine were obtained, and in 1923 Brauns²⁰⁵ prepared acetofluoroglucose. Brauns has extended these reactions to many of the reducing sugars and has measured the optical rotations of the acetohalogen sugars with high precision. He has deduced a relationship between these rotation values and the atomic dimensions of the halogens.²⁰⁶

The problem of obtaining the isomeric α -glycosides by the Koenigs and Knorr reaction was partially solved by Fischer and von Mechel²⁰⁷

²⁰² Koenigs and Knorr, *Ber.*, **34**, 978 (1901).

²⁰³ Colley, *Compt. rend.*, **76**, 436 (1873).

²⁰⁴ Fischer, *Ber.*, **44**, 1899 (1911).

²⁰⁵ Brauns, *J. Am. Chem. Soc.*, **45**, 833 (1923).

²⁰⁶ Brauns, *Bur. Standards J. Research*, **7**, 573 (1931); summarizing paper.

²⁰⁷ Fischer and v. Mechel, *Ber.*, **49**, 2813 (1916).

(1916) when they found that a mixture of glycosides was obtained when quinoline was substituted for silver carbonate in this reaction. What appears to be the best method for effecting this result was introduced by Pacsu²⁰⁸ (1928), who found that β -methylglucoside tetraacetate produced a nearly quantitative yield of the α -glucoside acetate on heating with titanium tetrachloride in chloroform solution. This method is reminiscent of the transformation of β -sugar acetates to the α -forms on heating with zinc chloride in acetic anhydride solution.

DISACCHARIDE STRUCTURE

Introduction. The term oligosaccharide has been suggested by Freudenberg to denote those polysaccharides which have a definitely known number of component molecular units. The oligosaccharides include the di-, tri-, and tetrasaccharides. Only the more common of the naturally occurring oligosaccharides have had their structure elucidated. Many more undoubtedly exist as constituents of those glycosides that produce several sugars on hydrolysis. A few such rare sugars have been isolated and characterized. A number of synthetic oligosaccharides have been prepared which are not identical with any as yet found in nature. The oligosaccharides are glycosidic condensation products of the monosaccharides, a second molecule of sugar acting as the alcohol, and when hydrolyzed the simple sugars are released and may be identified. Most of the oligosaccharides crystallize as hydrates.

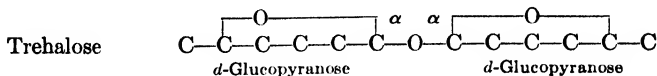
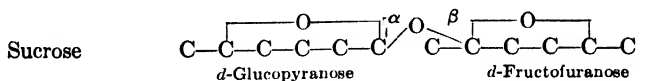
The structure of the common disaccharides will first be considered. The problems arising in the elucidation of the structure of the disaccharides may be classified under the following heads: (1) identification of the component sugars; (2) which of the component sugars is the alcohol portion; (3) the stereochemical nature (α - or β -) of the glycosidic linkage; (4) which carbon of the alcohol portion is concerned in the glycosidic union; and (5) the ring structure of each of the component sugars. The results of these studies have shown that the common disaccharides fall into three classes as regards the point of glycosidic union: (1) those linked through the reducing groups of each component; (2) those linked to carbon four of the alcohol portion, or the C₄-disaccharides; (3) those linked to carbon six of the alcohol portion, or the C₆-disaccharides. Furthermore, *d*-glucose is the alcohol portion of each. These structures are tabulated in Table VI.

In attacking the problems presented by disaccharide structure, the nature of the component sugars was the problem most readily solved. The stereochemical nature (α - or β -) of the glycosidic linkage was deter-

²⁰⁸ PACSU, *Ber.*, **61**, 137, 1513 (1928).

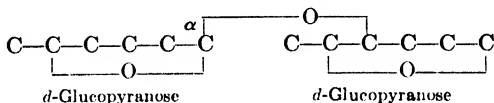
TABLE VI
STRUCTURE OF THE COMMON DISACCHARIDES

(1) Disaccharides linked through the reducing groups

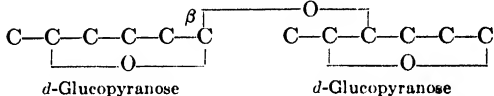


(2) C_4 -Disaccharides

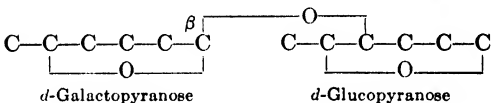
Maltose



Cellobiose

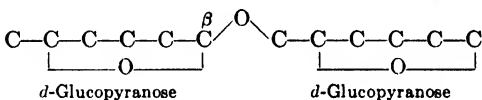


Lactose

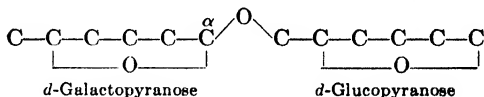


(3) C_5 -Disaccharides

Gentiobiose



Melibiose



mined by means of enzymic studies and by a consideration of the optical rotations involved; some of these results have not been entirely decisive. The remaining structural problems were solved by methylation methods. These consisted in methylating all free hydroxyl groups in the molecule, hydrolyzing, and ascertaining the linkage by the nature of the hydrolytic products obtained. This general procedure falls under the head of structural determination by degradative methods. The classical methods of the organic chemist then require that this structure be confirmed by synthesis from intermediates of known structure through controlled reactions.

The methylation of a disaccharide is quite a different problem from the methylation of a monosaccharide. The Fischer method for glycoside formation is inapplicable to a disaccharide because of the hydrolysis produced by the methanol solution of hydrogen chloride. The vigorous oxidizing action of silver oxide makes the Purdie methylation process

unsuitable for the methylation of a free reducing sugar. The methyl sulfate-alkali procedure was successfully adapted to disaccharide methylation by Haworth. Maquenne²⁰⁹ (1905) had used methyl sulfate for β -glucoside formation, and Haworth adopted the technique of Maquenne for the preliminary formation of a glycoside at low temperatures before using the more stringent conditions required for the remaining hydroxyl groups. A final methylation by the Purdie method was then generally used to ensure complete methylation.

Methylation Reference Compounds. In determining the nature of the hydrolytic products of the methylated disaccharides, a number of reference substances are of importance and will be first considered.

Tetramethylglucopyranose. *n*-Tetramethylglucose or tetramethylglucopyranose has been discussed previously, and as it crystallizes with ease its isolation never presented any difficulty.

Tetramethylgalactopyranose. This sugar was first prepared by Irvine and Cameron²¹⁰ (1904) as a syrup and characterized as its crystalline β -methyltetramethylgalactoside. A more useful characterizing derivative is the readily crystallized anilide of this sugar. The hydrazones of the methylated sugars are syrups and are of no value as derivatives, but Irvine and McNicoll²¹¹ (1910) showed that the anilide was frequently a crystallizable substance, especially for tetramethylgalactose. The (1,5-) lactol nature of this sugar was demonstrated when Haworth, Hirst, and Jones²¹² (1927) isolated crystalline *l*-arabotrimethoxyglutaromethylamide by the nitric acid oxidation of tetramethylgalactose. Schlubach and Moog²¹³ (1923) crystallized the free sugar, and in some of Haworth's later work the methylated sugar was isolated directly in crystalline form.

Tetramethylfructopyranose. *n*-Tetramethylfructose, a beautifully crystalline sugar, was first prepared by Purdie and Paul²¹⁴ (1907) by the methylation of that complex syrupy mixture of fructosides obtained by E. Fischer²¹⁵ (1895) through the action of methanol and hydrogen chloride upon fructose. The preparation of crystalline β -methylfructoside by Hudson and Brauns²¹⁶ (1916) provided a superior source for the sugar, and its preparation by the methylation and subsequent hydrolysis of this glycoside was reported by Irvine and Patterson²¹⁷

²⁰⁹ Maquenne, *Bull. soc. chim.*, [3] **33**, 469 (1905).

²¹⁰ Irvine and Cameron, *J. Chem. Soc.*, **85**, 1071 (1904).

²¹¹ Irvine and McNicoll, *ibid.*, **97**, 1449 (1910).

²¹² Haworth, Hirst, and Jones, *ibid.*, 2428 (1927).

²¹³ Schlubach and Moog, *Ber.*, **56**, 1957 (1923).

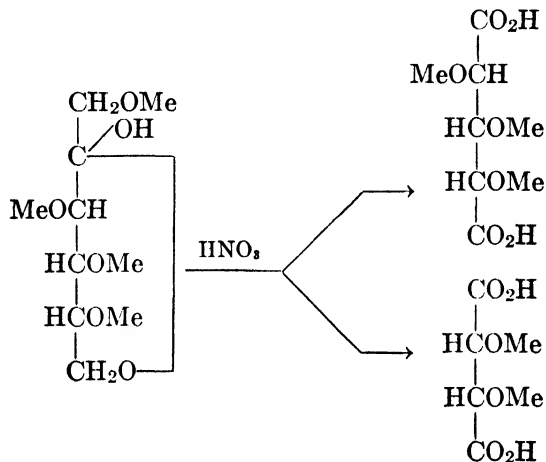
²¹⁴ Purdie and Paul, *J. Chem. Soc.*, **91**, 289 (1907).

²¹⁵ Fischer, *Ber.*, **28**, 1145 (1895).

²¹⁶ Hudson and Brauns, *J. Am. Chem. Soc.*, **38**, 1216 (1916).

²¹⁷ Irvine and Patterson, *J. Chem. Soc.*, **121**, 2146 (1922).

(1922). The pyranose or (2,6-) ring structure for this sugar was established by Haworth and Hirst²¹⁸ (1926) by the isolation of *d*-arabotri-methoxyglutaric acid and *i*-dimethoxysuccinic acid as their crystalline diamides from the nitric acid oxidation of *n*-tetramethylfructose.



2,3,6-Trimethylglucose. The trimethylglucose (I) having this structure was prepared in crystalline form by Denham and Woodhouse²¹⁹ (1914) as a hydrolytic product of methylated cellulose (p. 1552). These workers²²⁰ (1917) considered that the second position was occupied because the substance formed no osazone, and that the third carbon was methylated because, on cyanohydrin formation, demethylation occurred with the formation of a dimethyl lactone (II). Haworth and Hirst²²¹ (1921) showed that the sugar was convertible into *n*-tetramethylglucose (III) on further methylation and hydrolysis. A useful derivative of this trimethylglucose was obtained by Schlubach and Moog²²² (1923), when they isolated its β -methylglycoside in crystalline form. Further support for the third position carrying a methyl group was afforded by the isolation of this trimethylglucose from the hydrolysis products of methylated lactose by Haworth and Leitch²²³ (1918). Since Ruff and Ollendorf²²⁴ (1900) had obtained an osazone (VII) from the disaccharide (VI) resulting from the degradation of lactose by one carbon atom, then position three must be open in lactose. Good proof

²¹⁸ Haworth and Hirst, *ibid.*, 1858 (1926).

²¹⁹ Denham and Woodhouse, *ibid.*, **105**, 2357 (1914).

²²⁰ Denham and Woodhouse, *ibid.*, **111**, 244 (1917).

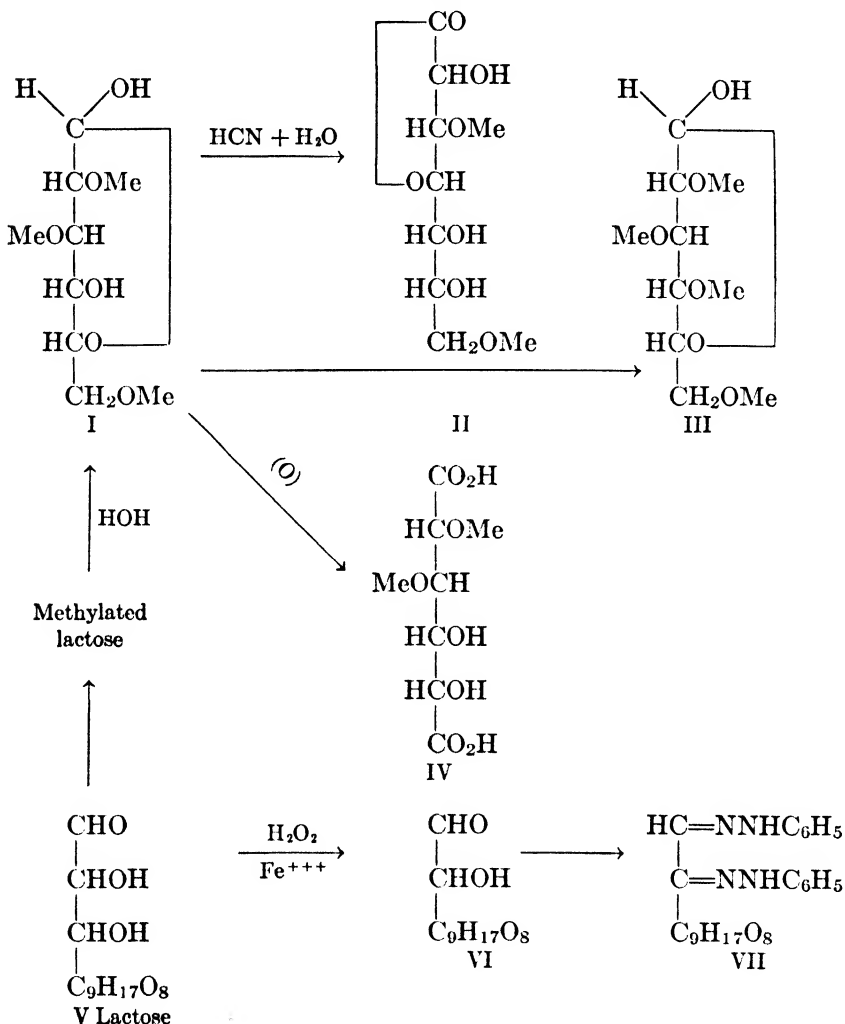
²²¹ Haworth and Hirst, *ibid.*, **119**, 193 (1921).

²²² Schlubach and Moog, *Ber.*, **56**, 1957 (1923).

²²³ Haworth and Leitch, *J. Chem. Soc.*, **113**, 197 (1918).

²²⁴ Ruff and Ollendorf, *Ber.*, **33**, 1806 (1900).

that the sixth position was occupied was provided by Irvine and Hirst²²⁵ (1922), who obtained a crystalline lead salt of a dimethylsaccharic acid (IV) on nitric acid oxidation of this trimethylglucose.

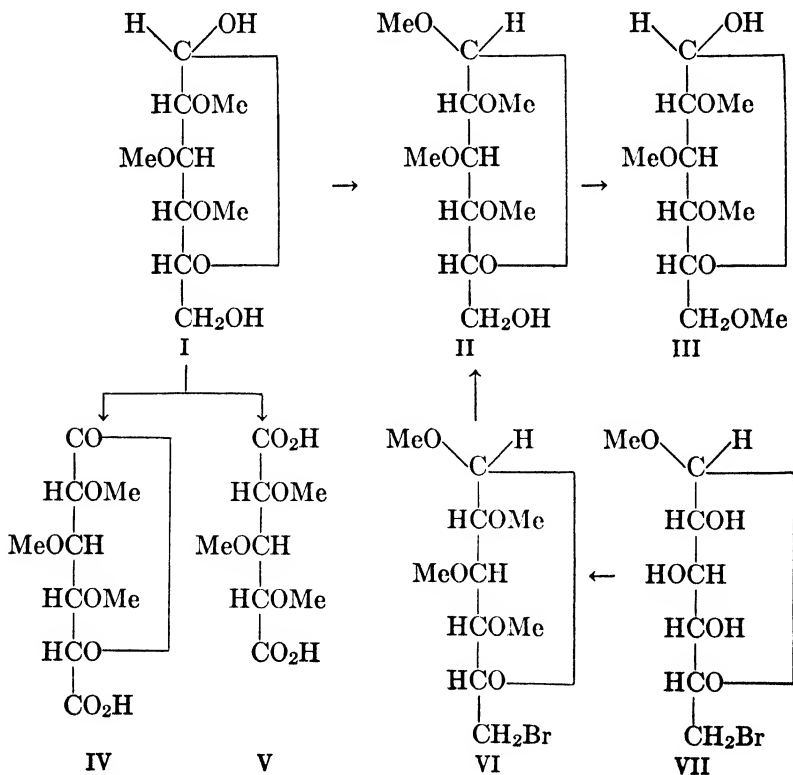


2,3,4-Trimethylglucose. This syrupy sugar (I) was first isolated as its α -methylglycoside by Purdie and Irvine²²⁶ (1903) through the partial methylation of α -methylglucoside. These workers converted it to *n*-tetramethylglucose (III) by further methylation and hydrolysis.

²²⁵ Irvine and Hirst, *J. Chem. Soc.*, **121**, 1213 (1922).

²²⁶ Purdie and Irvine, *ibid.*, **83**, 1021 (1903).

The free sugar was obtained by Purdie and Bridgett²²⁷ (1903). It is best characterized as its crystalline β -methylglycoside (II) described by Irvine and Oldham²²⁸ (1921). Irvine and Dick²²⁹ (1919) showed that the sixth position was open by obtaining a trimethylsaccharic acid (IV) on nitric acid oxidation. Conclusive proof of the structure of this substance was given by Irvine and Oldham²³⁰ (1925) by obtaining its crystalline β -methylglycoside (II) on methylation of the crystalline β -methylglucoside-6-bromohydrin (VII) of E. Fischer and subsequent hydrolysis of the bromine atom. This structure was confirmed by Charlton, Haworth, and Herbert²³¹ (1931) by obtaining the crystalline methylamide of *i*-xylotrimethoxyglutaric acid (V) and the crystalline methyl ester of 2,3,4-trimethylsaccharolactone (IV) on nitric acid oxidation.



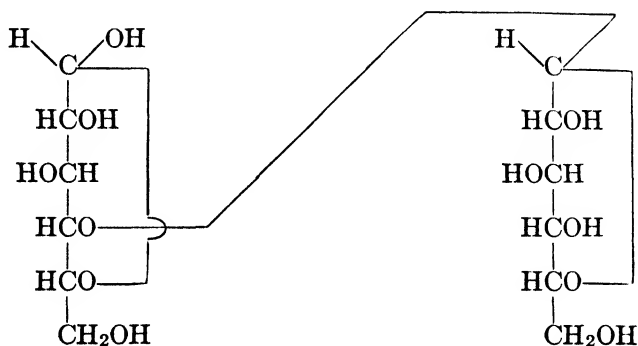
²²⁷ Purdie and Bridgett, *ibid.*, **83**, 1037 (1903).

²²⁸ Irvine and Oldham, *ibid.*, **119**, 1744 (1921).

²²⁹ Irvine and Dick, *ibid.*, **115**, 593 (1919).

²³⁰ Irvine and Oldham, *ibid.*, **127**, 2729 (1925).

²³¹ Charlton, Haworth, and Herbert, *ibid.*, 2855 (1931).

Determination of the Structures of the Common Disaccharides.*Maltose (4-d-Glucopyranosyl- α -d-glucopyranoside).*

Maltose was isolated by de Saussure²³² (1819) and is produced along with dextrans by the diastatic degradation of starch. It is reducing and produces two moles of *d*-glucose on hydrolysis. Its hydrolytic enzyme is maltase, and the sugar is therefore considered to possess an α -disaccharide linkage. E. Fischer had classified glycosides on the basis of enzyme specificity, the α -form being the one hydrolyzed by maltase and the β -isomer the one split by emulsin. This useful principle is not always of general application.

As early as 1905, Purdie and Irvine²³³ methylated maltose by the silver oxide method. Although oxidation occurred during the methylation process, they succeeded in isolating crystalline *n*-tetramethylglucose as a hydrolytic product of their methylated substance. Haworth and Leitch²³⁴ (1919) obtained a completely methylated and unoxidized maltose structure by the methyl sulfate procedure, and on hydrolysis of their methylheptamethylmaltoside they verified the results of Purdie and Irvine by obtaining *n*-tetramethylglucose. The second hydrolytic product was isolated in crystalline form and identified as 2,3,6-trimethylglucose in 1926 (Irvine and Black;²³⁵ Cooper, Haworth, and Peat²³⁶).

The isolation of 2,3,6-trimethylglucose as a hydrolytic product of a disaccharide does not definitely complete the structural determination, since the glucose molecule in the reducing portion might exist in either the pyranose or furanose form. It is necessary to prove that one of the two positions, four or five, is involved in the glycosidic linkage and

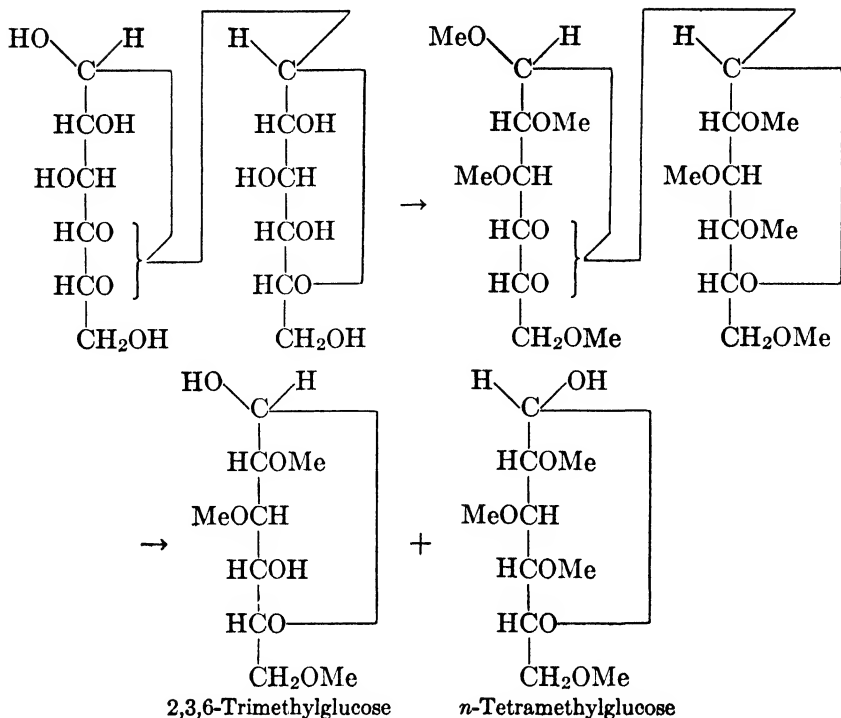
²³² de Saussure, *Ann. chim. phys.*, [2] **11**, 379 (1819).

²³³ Purdie and Irvine, *J. Chem. Soc.*, **87**, 1022 (1905).

²³⁴ Haworth and Leitch, *ibid.*, **115**, 809 (1919).

²³⁵ Irvine and Black, *ibid.*, 862 (1926).

²³⁶ Cooper, Haworth, and Peat, *ibid.*, 876 (1926).



the other is involved in the lactol structure. Definite allocation of the disaccharide linkage was made by Haworth and Peat²³⁷ (1926), who eliminated the troublesome ring in the reducing portion of the maltose molecule through oxidation to the bionic acid. Calcium maltobionate was methylated to form methyl octamethylmaltobionate, and on acid hydrolysis this yielded crystalline *n*-tetramethylglucose and 2,3,5,6-tetramethylgluconic acid, isolated as its crystalline phenylhydrazide (p. 1465). The structure of the latter follows from its previous formation by the hypobromite oxidation of tetramethylglucofuranose.

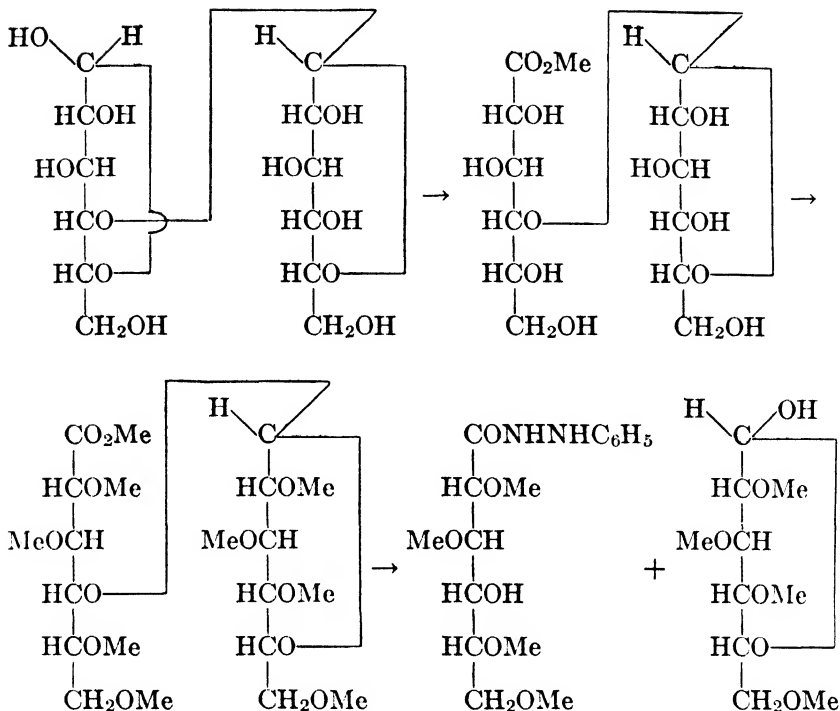
Cellulose (4-d-glucopyranosyl-β-d-glucopyranoside).

Skraup and König²³⁸ (1901) obtained this sugar in crystalline form by the saponification of its crystalline octaacetate, which had been prepared previously by Franchimont²³⁹ (1879) from the acetolysis of cellulose with acetic anhydride and sulfuric acid. The free sugar is readily obtained crystalline from the saponification of its acetate by

²³⁷ Haworth and Peat, *ibid.*, 3094 (1926).

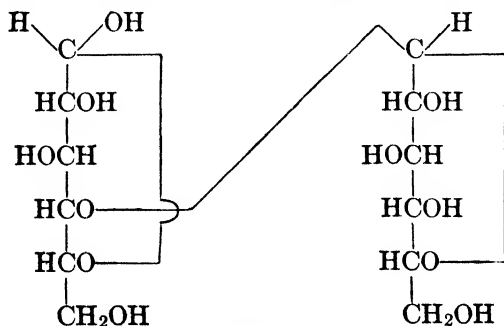
²³⁸ Skraup and König, *Ber.*, **34**, 1115 (1901).

²³⁹ Franchimont, *Ber.*, **12**, 1941 (1879).



sodium ethylate according to Zemplén²⁴⁰ (1926). As the best evidence indicates that this sugar is preformed in the cellulose molecule its structure is of fundamental importance. It is reducing and is hydrolyzed by acid or by emulsin into two moles of glucose. Accordingly it has a β -disaccharide configuration.

It differs from maltose only in its glycosidic configuration, and

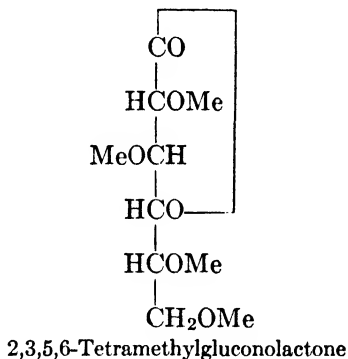


Cellobiose

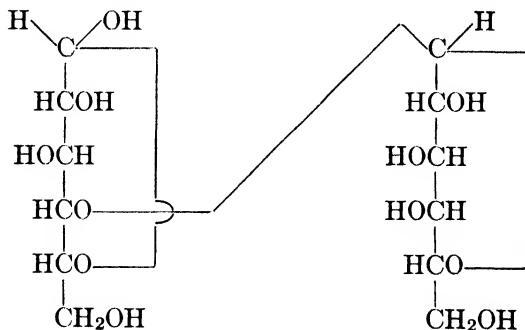
²⁴⁰ Zemplén, *Ber.*, **59**, 1258 (1926); Zemplén, Gerecs, and Hadácsy, *Ber.*, **69**, 1827 (1936).

similar methylation procedures were used to prove this point. Crystalline α -cellobiose octaacetate was simultaneously deacetylated and methylated with methyl sulfate and alkali by Haworth and Hirst²⁴¹ (1921) to form a crystalline methylheptamethylcellobioside, which on hydrolysis produced crystalline *n*-tetramethylglucose and the crystalline 2,3,6-trimethylglucose of Denham and Woodhouse.

As with maltose, these results limited the disaccharide linkage to carbon atoms four or five of the glucose molecule. Evidence for the selection of carbon four was given by Haworth, Long, and Plant²⁴² (1927). Cellobiose was oxidized to calcium cellobionate, and this on complete methylation produced methyl octamethylcellobionate. Hydrolysis of the latter produced *n*-tetramethylglucose and 2,3,5,6-tetramethylgluconolactone, which in this case was isolated in crystalline condition (m. p. 26–27°) and also as its crystalline phenylhydrazide.



Lactose (4-*d*-Glucopyranosyl- β -*d*-galactopyranoside).



Lactose or sugar of milk is one of the most common of the disaccharides. It is probably present in the milk of all mammals and is pre-

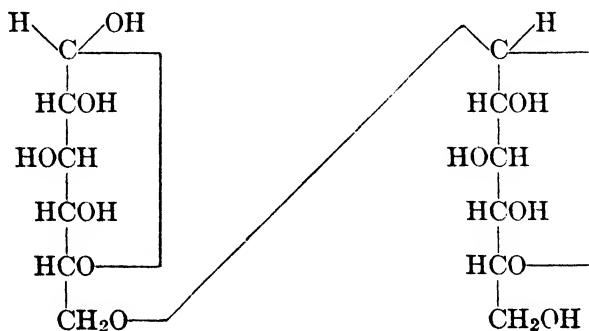
²⁴¹ Haworth and Hirst, *J. Chem. Soc.*, **119**, 193 (1921).

²⁴² Haworth, Long, and Plant, *ibid.*, 2809 (1927).

pared commercially in considerable quantity from cow's milk. It is readily crystallized as the α -hydrate, but the β -form is also known, and this form is sweeter and more soluble than the α -isomer. Two disaccharides isomeric with lactose have been isolated from human milk by Polonovski and Lespagnol²⁴³ (1930; 1931). Lactose was recorded in 1633 by the physician Fabriccio Bartoleti,²⁴⁴ in Bologna, although his publication would seem to indicate that it was previously known. It produces one mole of glucose and one mole of galactose on acid hydrolysis and is a reducing sugar. It is not hydrolyzed by maltase but is hydrolyzed by lactase. The recent extensive investigations of Helferich have shown that lactase is identical with the β -glucosidase of emulsin. The sugar thus possesses a β -configuration, and this is supported by the calculations of Hudson on its optical rotatory power.

The structure of lactose was proved by Haworth in a manner similar to that used for the preceding sugars. Haworth and Leitch²⁴⁵ (1918) prepared and hydrolyzed crystalline methylheptamethylactoside to produce *n*-tetramethylgalactose, isolated as the crystalline anilide, and the crystalline 2,3,6-trimethylglucose of Denham and Woodhouse. In 1927, Haworth and Long²⁴⁶ oxidized lactose to barium lactobionate and methylated this product to form the syrupy methyl octamethylactobionate which on hydrolysis produced *n*-tetramethylgalactose, isolated in this case in crystalline condition, and 2,3,5,6-tetramethylgluconolactone, isolated in crystalline form and also as its crystalline phenylhydrazide.

Gentiobiose (6-d-Glucopyranosyl- β -d-glucopyranoside).



²⁴³ Polonovski and Lespagnol, *Compt. rend. soc. biol.*, **104**, 553 (1930); *Compt. rend.*, **192**, 1319 (1931).

²⁴⁴ Bartoleti, "Methodus in dyspnoeam—," 400 (1633); cf., von Lippmann, "Geschichte des Zuckers," 2nd ed., Springer, Berlin (1929), p. 688.

²⁴⁵ Haworth and Leitch, *J. Chem. Soc.*, **113**, 188 (1918).

²⁴⁶ Haworth and Long, *ibid.*, **544** (1927).

A. Meyer²⁴⁷ (1882) isolated a crystalline non-reducing trisaccharide from the gentian root (*Gentiana lutea*) and named it gentianose. Bourquelot and Hérissé²⁴⁸ (1901) found that weak acids split off one mole of fructose and leave a disaccharide which they named gentiobiose. This disaccharide is reducing and produces two moles of glucose on further hydrolysis. According to the Fischer principle, gentiobiose has a β -linkage, since it is hydrolyzed by emulsin. The classical work of Bourquelot on the reversal of hydrolytic enzyme reactions has provided a method for the synthesis of gentiobiose²⁴⁹ (1913) from glucose and emulsin.

It is now agreed that gentiobiose is the disaccharide component of amygdalin, the glycoside of bitter almonds. This solution was reached independently in 1924 by a number of workers. By the application of the isorotation rules Hudson²⁵⁰ calculated that the biose of amygdalin must be gentiobiose. This was confirmed synthetically by Zemplén,²⁵¹ Kuhn and Sobotka,²⁵² and by Campbell and Haworth,²⁵³ the attachment of the sugar being effected by acetobromogentiobiose. Amygdalin is now known to be *l*-mandelonitrile- β -gentiobioside. Gentiobiose octaacetate may be prepared from acetylated amygdalin by catalytic hydrogenation (Bergmann and W. Freudenberg,²⁵⁴ 1929) and further acetylation.

Haworth and Wylam²⁵⁵ (1923) obtained a crystalline methylheptamethylgentiobioside by the complete methylation of gentiobiose, and this on acid hydrolysis produced crystalline *n*-tetramethylglucose and 2,3,4-trimethylglucose, identified as its crystalline β -methylglycoside. This indicated that the disaccharide linkage was on carbon six and the sugar ring on carbon five, or *vice versa* with the probability in favor of the former. Later synthetic experiments decided the carbon six disaccharide linkage.

The synthetic work of Helferich²⁵⁶ and his collaborators (1924–1926) has confirmed the structure of gentiobiose as 6-glucosidoglucose. Helferich found that triphenylmethyl chloride (called by him trityl chloride) reacted preferentially with primary alcohol groups. Glucose reacted with trityl chloride to form a 6-monotrityl ether, the allocation

²⁴⁷ Meyer, *Z. physiol. Chem.*, **6**, 135 (1882).

²⁴⁸ Bourquelot and Hérissé, *Compt. rend.*, **132**, 571 (1901).

²⁴⁹ Bourquelot, Hérissé, and Coirre, *ibid.*, **157**, 732 (1913).

²⁵⁰ Hudson, *J. Am. Chem. Soc.*, **46**, 483 (1924).

²⁵¹ Zemplén and Kunz, *Ber.*, **57**, 1357 (1924).

²⁵² Kuhn and Sobotka, *Ber.*, **57**, 1767 (1924).

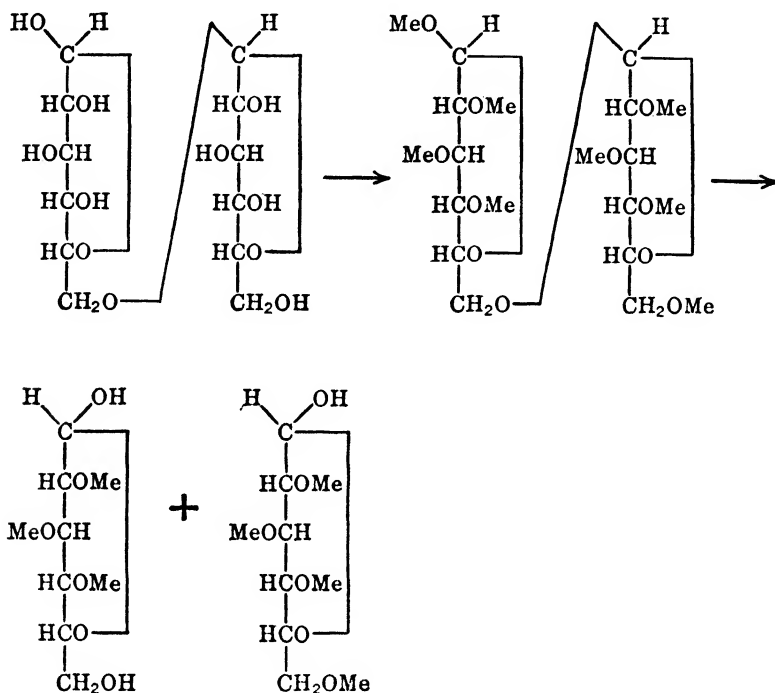
²⁵³ Campbell and Haworth, *J. Chem. Soc.*, **125**, 1337 (1924).

²⁵⁴ Bergmann and W. Freudenberg, *Ber.*, **62**, 2783 (1929).

²⁵⁵ Haworth and Wylam, *J. Chem. Soc.*, **123**, 3120 (1923).

²⁵⁶ Helferich, *Z. angew. Chem.*, **41**, 871 (1928); summarizing paper.

being proved by treatment with phosphorus pentabromide to form derivatives of 6-bromoglucose. Fischer and Armstrong²⁵⁷ (1902) had previously obtained derivatives of this substance, and Fischer and Zach²⁵⁸ (1912) had determined that the bromine was on the terminal



carbon atom by reduction to the methylpentose isorhamnose. Helferich acetylated 6-tritylglucose and then removed the trityl group by mild treatment with hydrogen bromide, thus obtaining a glucose structure with only the sixth position open. Reaction with acetobromoglucose produced gentiobiose octaacetate, the β -configuration being also confirmed by the well-established fact that acetobromoglucose produces only β -glycosides.

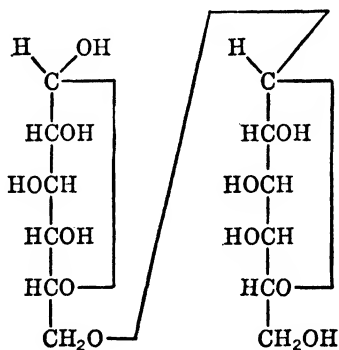
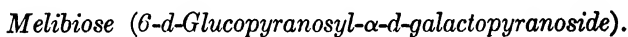
By analogous synthetic methods, Helferich and Rauch²⁵⁹ (1927) showed that the rare crystalline disaccharide primeverose was 6-xylosido-glucose. This sugar had been obtained from primeverin²⁶⁰ (1912), a glycoside isolated from the roots of *Primula officinalis*.

²⁵⁷ Fischer and Armstrong, *Ber.*, **35**, 833 (1902).

²⁵⁸ Fischer and Zach, *Ber.*, **45**, 3761 (1912).

²⁵⁹ Helferich and Rauch, *Ann.*, **455**, 168 (1927).

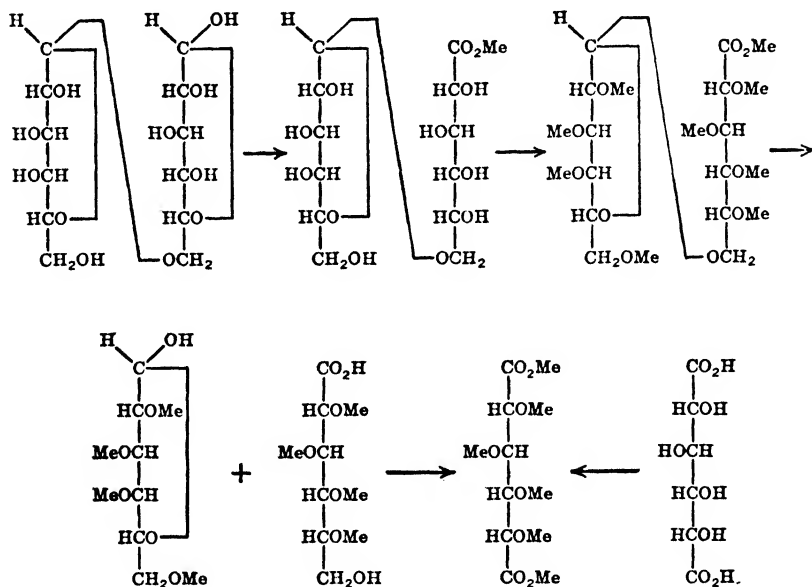
²⁶⁰ Goris, Maseré, and Vischniac, *Bull. sci. pharmacol.*, **19**, 577 (1912); Goris and Vischniac, *Bull. soc. chim.*, [4] **27**, 258 (1920).



²⁶¹ Johnston, *J. prakt. Chem.*, **29**, 485 (1843).

Loiseau²⁶² (1876) and from cottonseed hulls by Ritthausen²⁶³ (1884). Its complete hydrolytic products are glucose, fructose, and galactose, but the fructose may be selectively hydrolyzed by invertase, leaving the reducing disaccharide melibiose. The latter observation was first made by M. Berthelot²⁶⁴ (1856) but was discredited, and the crystalline melibiose was not obtained until 1899 by Bau.²⁶⁵ The best evidence at present points to the disaccharide linkage being of the α -configuration.

Haworth, Loach, and Long²⁶⁶ (1927) oxidized melibiose to the bionic acid and completely methylated this to the syrupy methyl octamethylmelibionate. On acid hydrolysis this produced *n*-tetramethylgalactose, isolated as the crystalline anilide, and a syrupy tetramethylgluconic acid. Nitric acid oxidation of the latter yielded a crystalline methyl tetramethylsaccharate, previously obtained by Karrer and Peyer²⁶⁷ (1922) from saccharic acid. Consequently the tetramethylgluconic acid was 2,3,4,5-tetramethylgluconic acid, and the constitution of melibiose was proved to be 6-galactosidoglucose.



²⁶² Loiseau, *Compt. rend.*, **82**, 1058 (1876); *Ber.*, **9**, 732 (1876).

²⁶³ Ritthausen, *J. prakt. Chem.*, [2] **29**, 351 (1884).

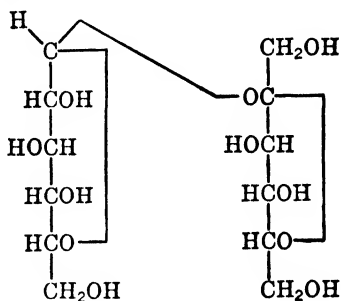
²⁶⁴ Berthelot, *Ann. chim. phys.*, [3] **46**, 66 (1856).

²⁶⁵ Bau, *Wochschr. Brau.*, **16**, 397 (1899); [*Chem. Zentr.* (II), 526 (1899)].

²⁶⁶ Haworth, Loach, and Long, *J. Chem. Soc.*, 3146 (1927).

²⁶⁷ Karrer and Peyer, *Helv. Chim. Acta*, **5**, 577 (1922).

Sucrose (1- α -d-glucopyranosyl- β -d-fructofuranoside).



Sucrose or common table sugar has long been known. This sugar is very widely distributed in the plant kingdom and is apparently the transport form of carbohydrate for many plants, as *D*-glucose is for the animal. The main commercial sources are the sugar cane and the sugar beet, and the final product is sold to the consumer in a very high degree of purity.

Sucrose is a non-reducing disaccharide, and this fact solves immediately the points of attachment of its component sugars as being through their glycosidic carbon atoms. It is hydrolyzed with great ease by dilute acids and also by its enzyme invertase to produce one mole each of glucose and fructose. The stereochemical nature of the two glycosidic linkages has not been determined definitely, but the evidence points to an α -linkage for the glucose component and to a β -linkage for the fructose portion. From a study of the kinetics of sucrose hydrolysis by concentrated solutions of invertase at low temperatures, Hudson²⁶⁸ (1909) considered that the glucose component was α -glucose and that the fructose component was a new form of fructose.

Purdie and Irvine²⁶⁹ (1903) isolated *n*-tetramethylglucose on hydrolysis of a methylated product obtained by the methylation of sucrose by the Purdie silver oxide method. Haworth²⁷⁰ (1915) prepared a completely methylated octamethylsucrose, succeeding in this by using successively the methyl sulfate and Purdie alkylation procedures. Sucrose tends to stop methylation at the heptamethyl stage, the eighth methyl group entering with difficulty. Haworth and Law²⁷¹ (1916) hydrolyzed this octamethylsucrose under the mildest possible conditions and separated the hydrolytic products by high vacuum dis-

²⁶⁸ Hudson, *J. Am. Chem. Soc.*, **31**, 655 (1909).

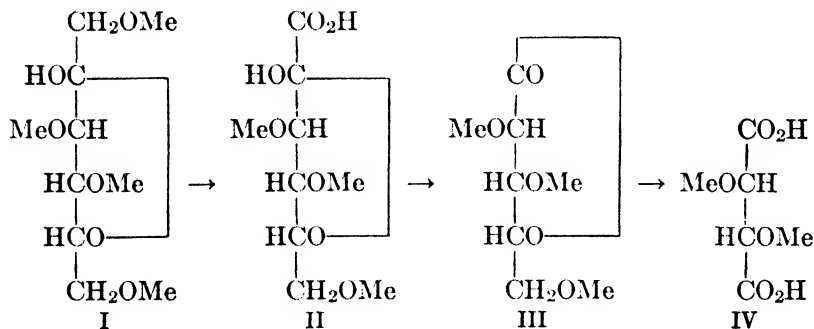
²⁶⁹ Purdie and Irvine, *J. Chem. Soc.*, **83**, 1021 (1903).

²⁷⁰ Haworth, *ibid.*, **107**, 12 (1915).

²⁷¹ Haworth and Law, *ibid.*, **109**, 1314 (1916).

tillation. Crystalline *n*-tetramethylglucose was obtained from the higher-boiling fraction. The lower-boiling fraction proved to be a dextrorotatory tetramethyl hexose which was not identical with the crystalline and highly levorotatory *n*-tetramethylfructose of Purdie and Paul. Consequently, the fructose component of sucrose must possess a different ring structure from that of ordinary fructose. The ring structure of this so-called γ -fructose was found to be of the furanose (2,5-) type by Avery, Haworth, and Hirst²⁷² (1927). Haworth²⁷³ (1920) found that the readily obtainable heptamethylsucrose contained a fully methylated fructose portion. Heptamethylsucrose was accordingly used as the best source of γ -tetramethylfructose.

In the work of Avery, Haworth, and Hirst, the syrupy γ -tetramethylfructose (I), obtained by the mild acid hydrolysis of heptamethylsucrose, was oxidized with nitric acid and the oxidation product (II) isolated as the ethyl ester. This substance was a lactol or glucosonic acid which could be methylated to form a non-reducing glycoside, and this in turn produced a crystalline amide. Oxidation of the lactol acid (II) with barium permanganate in acid solution yielded the crystalline (m. p. 29°) trimethyl- γ -*d*-arabonolactone (III). The enantiomorph of this had been previously obtained by Baker and Haworth²⁷⁵ (1925) from trimethyl-*l*-arabinofuranose. Nitric acid oxidation of this lactone yielded (levo)-dimethoxysuccinic acid (IV), characterized as its crystalline amide and methylamide. This very excellent oxidation work definitely characterizes the fructose component of sucrose as *d*-fructofuranose.



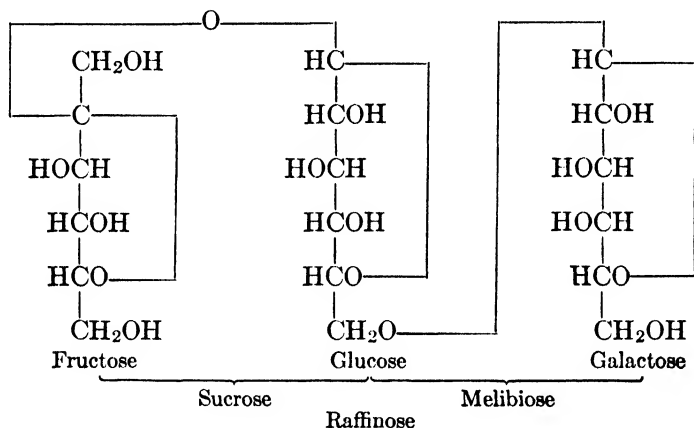
²⁷² Avery, Haworth, and Hirst, *ibid.*, 2308 (1927).

²⁷³ Haworth, *ibid.*, 117, 199 (1920).

²⁷⁵ Baker and Haworth, *ibid.*, 127, 365 (1925).

TRI- AND TETRASACCHARIDES

Raffinose is the most important of the trisaccharides and was methylated by Haworth, Hirst, and Ruell²⁷⁶ (1923) to the syrupy hendecamethylraffinose. On hydrolysis this produced tetramethylfructofuranose, tetramethylgalactopyranose, and 2,3,4-trimethylglucose. The second component was isolated as its crystalline anilide, and the third as its crystalline β -methylglycoside. This is in agreement with the work of Neuberg²⁷⁷ (1907), who showed that the trisaccharide is split enzymatically into one mole of sucrose and one mole of galactose. Since dilute acids produce melibiose and fructose, the constitution of raffinose follows.



The trisaccharide gentianose has been previously discussed under gentiobiose. Melezitose was isolated by Bonastre²⁷⁸ (1833) from the exudation of the larch. It occurs occasionally in honey. Melezitose is a non-reducing sugar, and mild acid hydrolysis produces one mole of glucose and one mole of turanose. The latter produces one mole each of glucose and fructose, on further hydrolysis. Turanose has long been known, but has only recently been crystallized.²⁷⁹ It is an isomer of sucrose in which fructose comprises the reducing portion.

An interesting sugar is the non-reducing tetrasaccharide stachyose isolated in crystalline condition by Schulze and von Planta²⁸⁰ (1890) from the tubers of the Japanese artichoke (*Stachys tuberosa*). Hydroly-

²⁷⁶ Haworth, Hirst, and Ruell, *ibid.*, **123**, 3125 (1923).

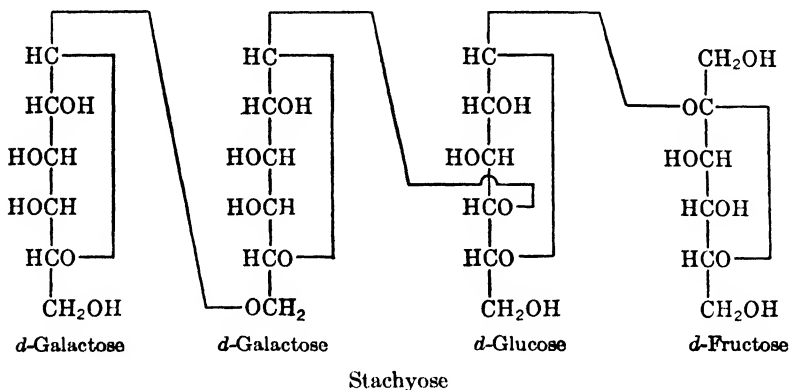
²⁷⁷ Neuberg, *Biochem. Z.*, **3**, 519 (1907).

²⁷⁸ Bonastre, *J. de Pharm.*, T. II, 19, 443 (1833).

²⁷⁹ Hudson, Brauns, and Pacsu, *Science*, **69**, 278 (1929).

²⁸⁰ v. Planta and Schulze, *Ber.*, **23**, 1692 (1890).

sis produces one mole of fructose, one of glucose, and two moles of galactose. Onuki²⁸¹ (1932; 1933) has produced evidence from methylation studies that stachyose can be represented by the following formula:



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²⁸¹ Onuki, *Sci. Papers Inst. Phys. Chem. Research* (Tokyo), **18**, 357 (1932); **20**, 201 (1933); [*Chem. Zentr.*, (II), 1007 (1932); (II), 367 (1933)].

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CHAPTER 17

CARBOHYDRATES II

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* Now with G. D. Searle and Co., Chicago, Ill.

SUBSTITUTED SUGARS

The preceding chapter has been concerned with the structures of the carbohydrates and the methods involved in determining them, and with certain of the reactions of the sugars. The present chapter will devote less attention to questions of structure and configuration, but will discuss certain substituted and derived sugars, and will consider some of their isomerizations and degradations.

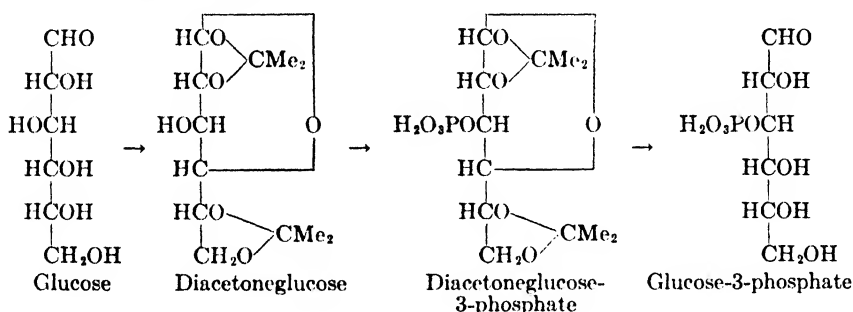
In many branches of organic chemistry, the original interest of the subject was either the elucidation of the structure of naturally occurring substances or an inquiry into biological processes. This is particularly true in respect to carbohydrate chemistry, and, although often obscured by the tremendous complexity of the field, the basic interest may be attributed to the extensive distribution in nature of carbohydrates and their derivatives and to their extremely important biological functions. The present discussion will consider the subject from this viewpoint, and will devote particular attention to those substances which are of biological as well as of chemical interest.

Esters. A number of carbohydrate derivatives which might be considered of minor chemical importance achieve a significance through their biological associations, and to this class belong many of the esters. Thus while the acetyl and benzoyl esters which have been previously discussed are only infrequently of biological interest, the phosphoric esters on the other hand are of extreme importance and of wide distribution in nature. Some of them have been discovered in muscle metabolism, several have been isolated from fermentation processes, to which they are essential, and a further group are constituents of the nucleic acids (p. 1011). The related glycerol phosphate is a constituent of certain lipides, and the likewise-related phosphoglyceric acid is found in the blood in significant amounts. These last two compounds have further important functions which will be discussed under fermentations.

From studies on fermentation and on muscle enzymes a number of phospho esters have been reported from time to time, but they have not all been authenticated since there is considerable difficulty in securing them in pure state. Three hexose esters for which the structures have been established are the Harden-Young diphosphate (fructose-1,6-diphosphate), the Robison monophosphate (glucose-6-phosphate), and the Neuberg ester (fructose-6-phosphate). Two additional fermentation esters which have been much less investigated are a mannose phosphate and a trehalose phosphate which yields the Robison ester on hydrolysis and must, therefore, be trehalose-6-monophosphate. In the pentose series, ribose-3- and 5-phosphates have been secured from yeast nucleic

acid degradations, but the 2-desoxyribose phosphate which must be the corresponding constituent of thymus nucleic acid has not as yet been isolated. In very recent years the great activity in the field of fermentation research has led to the isolation of the triose phosphates; dihydroxyacetone phosphate, and glyceraldehyde-2- and 3-phosphates.

Partly in connection with structural studies on the above esters, but mostly because of researches on the possible functions of the phosphoric esters in nature, a number of synthetic phosphates have been prepared. The procedure usually consists in the treatment of a partially substituted carbohydrate with phosphorus oxychloride in the presence of dry quinoline or pyridine or some aqueous alkali. The directing substituents are then removed; the product is isolated and may be purified by precipitation of its barium or calcium salt or by crystallization of its alkaloidal salts. As a typical example¹ there may be illustrated the synthesis of glucose-3-phosphate through the intermediary of diacetoneglucose (p. 1425):

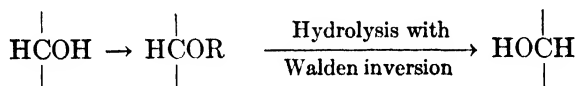


The method is general and requires only that the appropriate partially substituted derivative be available, and that removal of the directing substituents be possible under conditions which leave the phospho group intact. These requirements have been met in a number of instances, and in this manner there have been synthesized the Robison ester mentioned above, as well as glucose-1-, 3-, 4-, and 5-phosphates, leaving only the 2-phosphate unknown in the glucose series. Fructose-1- and 3-phosphates, galactose-6-phosphate, *d*-ribose-5-phosphate (and incidentally uridine- and inosine-5-phosphates), *d*-xylose-5-phosphate, and all three of the triose phosphates previously mentioned have been likewise synthesized. There has been reported the synthesis by less clear-cut procedures of the Harden-Young diphosphate and the Neuberg

¹ Levene and Meyer, *J. Biol. Chem.*, **53**, 431 (1922); Komatsu and Nodzu, *Mem. Coll. Sci. Kyoto Imp. Univ.*, **7**, Series A, 377 (1924); Nodzu, *J. Biochem. (Japan)*, **6**, 31 (1926); Raymond and Levene, *J. Biol. Chem.*, **83**, 619 (1929).

monophosphate. Attempts to synthesize *d*-xylose-3-phosphate resulted instead in the 5-phosphate, migration of the phospho group having apparently occurred during removal of the directing groups. This is the only recorded instance of non-enzymic migration of a phospho group, although migration of other substituents is not uncommon.

In the above syntheses it has been assumed that the phosphorylation was not attended by Walden inversion, and in this connection it is desirable to refer to Robinson's assumption² as to the origin of certain sugars in nature. In experiments on hydrolysis of sugar esters, particularly the tosyl * esters, it has been observed that frequently, although not invariably, there occurs a Walden inversion (pp. 197, 1844), resulting in the formation of a new sugar. Thus, considering only the carbon atom in question:



Robinson has suggested that in nature certain sugar esters such as the phosphates are formed, and are then similarly hydrolyzed with attendant Walden inversion, thus producing a new sugar. In this way glucose could yield glucose-4-phosphate and then on hydrolysis could give rise to galactose, thus accounting for the origin of this sugar. Against this ingenious explanation it should be observed that in all investigations thus far, enzymic dephosphorylation has not produced any sugar other than that originally phosphorylated. Although this does not exclude the possibility that some system actually exists in nature where this change takes place, the reaction has not as yet been demonstrated.

A second group of esters, owing their particular interest to their biological origin, are the sulfuric esters³ which occur as constituents of the mucoproteins and in certain seaweeds. In the mucoproteins the non-protein portion has been found to be a complex of glucuronic acid, acetic acid, sulfuric acid, and an aminohexose (chondrosamine or chitosamine). In these compounds the sulfuric acid is apparently attached to a hydroxyl of the hexosamine, but since this sulfuric linkage is most easily severed, the degradation products are all sulfate-free so the point of attachment has not been definitely established. A few synthetic sulfate esters have been prepared by a method analogous to that for the phosphate esters except that the phosphorus oxychloride is

² Robinson, *Nature*, **120**, 44 (1927).

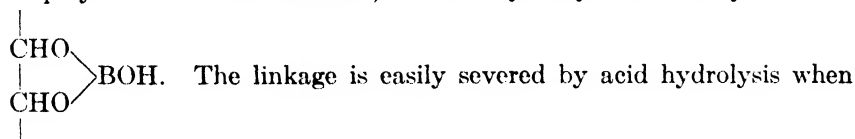
* "Tosyl" will be used throughout this chapter as an abbreviation for the *p*-toluene-sulfonyl group, and TIS will be used in formulas.

³ Levene, "Hexosamines and Mucoproteins," Longmans, Green, and Co., London (1925).

replaced by chlorosulfonic acid or sulfuryl chloride. In this manner there have been prepared derivatives of glucose-3-, 6-, and possibly 5-sulfates, and fructose-1- and 3-sulfates. Certain of these esters have been studied by Ohle and co-workers, who consider that the oxidation mechanism of fructose sulfuric (and phosphoric) esters affords insight into the biological processes of carbohydrate degradation.

A further group of naturally occurring esters is that of the tannins. These substances were reported to contain glucose, and, although this was disputed, it seems confirmed by the best available evidence. On the assumption that the small glucose content was due to the presence of several digalloyl residues, Fischer synthesized pentagalloyl- and penta-digalloylglucose and found them to be similar in properties to the natural galloyl tannins.^{3a} Another synthetic derivative, 1-galloylglucose, has been found to be identical in every respect with a natural product which is associated with the tannins, and a crystalline tannin has been isolated which is apparently a digalloylglucose. In these esters one or more of the carbohydrate hydroxyls are esterified with either gallic, $C_6H_2(OH)_3COOH$, or digallic acid, $HOCC_6H_2(OH)_2OCOC_6H_2(OH)_3$.

Leaving for the moment the naturally occurring esters, mention may be made of the borates, which have received attention recently. From a synthetic standpoint they are of particular interest because in them the substitution is frequently in positions which are different from those of the other common substituting groups. Metaboric acid is employed in the condensations, and two hydroxyls are usually involved



desired so that the preparation of new, partially substituted derivatives is facilitated.⁴

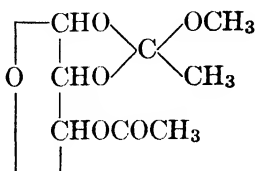
A final type of ester which needs consideration is that of the ortho-acetates. These were first discovered^{4a} in preparing a glycoside from acetobromorhamnose and methyl alcohol in the presence of silver carbonate. They differ from the normal glycosides usually produced by this reaction, being characterized by having one acetyl group resistant to even vigorous alkaline hydrolysis. On the other hand this acetyl and the methyl group are hydrolyzed by the mildest acid treatment, and

^{3a} Fischer and Freudenberg, *Ber.*, **45**, 915 (1912); Fischer and Bergmann, *Ber.*, **51**, 1760 (1918); *Ber.*, **52**, 829 (1919).

⁴ Brigl and Grüner, *Ann.*, **495**, 60 (1932); *Ber.*, **66**, 1977 (1933); *Ber.*, **67**, 1969 (1934); von Vargha, *Ber.*, **66**, 704, 1394 (1933).

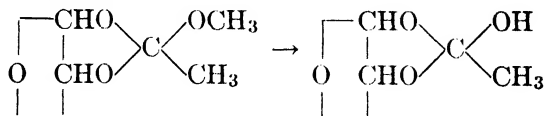
^{4a} Fischer, Bergmann, and Rabe, *Ber.*, **53**, 2385 (1920)

on this account they were considered to be furanosides, with one acetyl group unaccountably stabilized. The correct explanation was indicated by Braun⁵ from a study of ultra-violet absorption bands; the subject was then further studied by Freudenberg and, at about the same time, by Haworth. It was thus shown that one acetyl group is in the orthoacetate form, being attached to two of the sugar hydroxyls and to the methyl group.



It is of great interest that the formation of the orthoacetates is, for some unknown reason, facilitated by having *cis* hydroxyls on carbons two and three of the sugar, so that the orthoacetate is the dominant reaction product with the sugars falling in this category. It has also been observed, however, with sugars not belonging to this type, for example, in the disaccharide turanose. This sugar is of further interest since Pacsu⁶ claims to have secured the two orthoacetates which would be expected because a new asymmetric carbon has been created in the orthoacetate.

A further example of these derivatives has been provided by Isbell,⁷ who was able to hydrolyze the methyl group in heptaacetyl-4-glucosidomethylmannoside. The resultant compound differed from the ordinary glucosidomannose heptaacetates in that it exhibited no mutarotation, and this was interpreted by Isbell as being due to an orthoacetate structure.



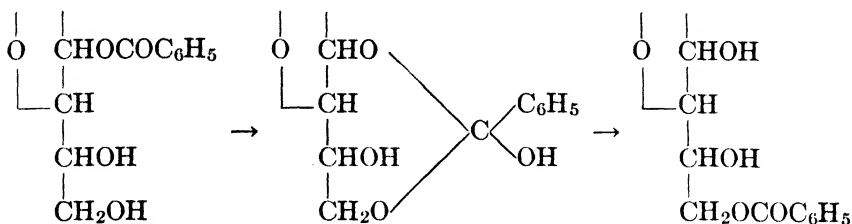
In connection with the orthoacetates there may be considered the migration of acyl substituents which has been observed, and has been assumed to pass through the ortho form as an intermediate. For example, the migration of the benzoyl group from position three to six

⁵ Braun, *Naturwissenschaften*, **18**, 393 (1930); *Ber.*, **63**, 1972 (1930); Freudenberg, *Naturwissenschaften*, **18**, 393 (1930); Freudenberg and Scholz, *Ber.*, **63**, 1969 (1930); Haworth, Hirst, and Miller, *J. Chem. Soc.*, 2469 (1929); Bott, Haworth, and Hirst, *ibid.*, 1393 (1930).

⁶ Pacsu, *J. Am. Chem. Soc.*, **54**, 3649 (1932); see, also, Goebel and Babers, *J. Biol. Chem.*, **110**, 707 (1935).

⁷ Isbell, *J. Research Nat. Bur. Standards*, **7**, 1115 (1931).

in monoacetoneglucose, for which reaction Josephson has provided some excellent data on rates, may be written as follows:



Similar mechanisms have been assumed for other migrations⁸ of this type. Acetyl groups, as well as benzoyl groups, have frequently been found to undergo such migrations. On the other hand only one migration of a phospho group in the sugars has been described. There seems to be no authenticated instance in the literature of any such migration of alkyl groups. A single example which is of considerable interest, but of a different type, is that reported by Ohle,⁹ who observed intermolecular migration of a tosyl group: a monotosyl derivative, on treatment with ammonia, was found to give rise to a ditosyl derivative, a part of the material having tosylated the remainder.

These migrations are of major importance in considerations of structure, and it is necessary to be extremely cautious in basing conclusions upon positions of the original substituents, which may have been altered by the subsequent reactions.

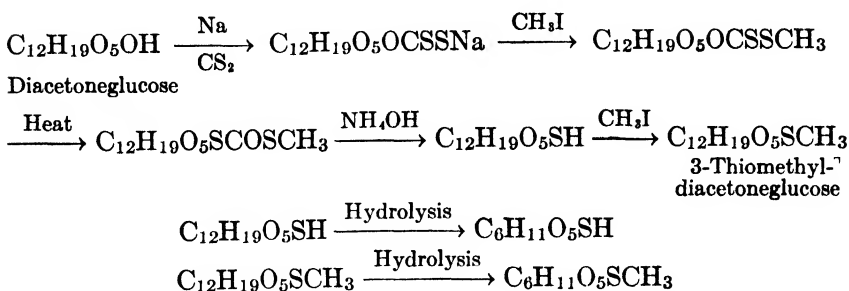
Thio Sugars. Turning from the esters of the sugars to other derivatives occurring in nature, mention may be made of the thio sugars. Apart from the fairly prevalent thioglucosides only one thio sugar from a natural source is known, and this is the thiomethylpentose which occurs in yeast. It is combined as an adenosine nucleoside, but its structure has not yet been established beyond question. Along synthetic lines there have been only two studies on sugars in which the thio group is non-glycosidic, that of Freudenberg on 3-thio- and 3-thio-methylglucose, and that of Raymond on certain sugars in which the thiomethyl group was substituted for the primary hydroxyl.

In the first of these investigations, diacetoneglucose was converted to the xanthogenate, which was methylated, isomerized by heating, hydrolyzed to the thiodiacetoneglucose, and re-methylated to thiomethyl-

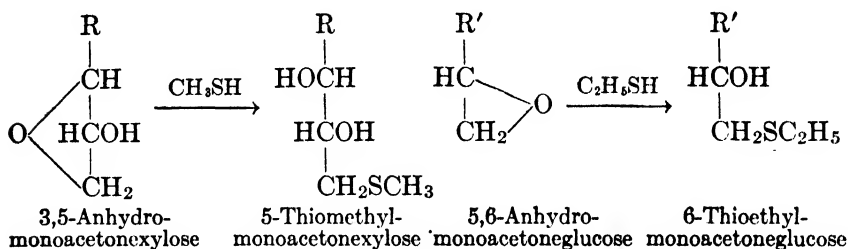
⁸ Helferich and Klein, *Ann.*, **455**, 173 (1927); Haworth, Hirst, and Teece, *J. Chem. Soc.*, 1405 (1930); 2858 (1931); Helferich and Müller, *Ber.*, **63**, 2142 (1930); Josephson, *Svensk Kem. Tid.*, **41**, 99 (1929); *Ber.*, **63**, 3089 (1930).

⁹ Ohle and Lichtenstein, *Ber.*, **63**, 2905 (1930).

diacetoneglucose. Acid hydrolysis removed the acetone groups, giving the free thio- or thiomethylhexose:



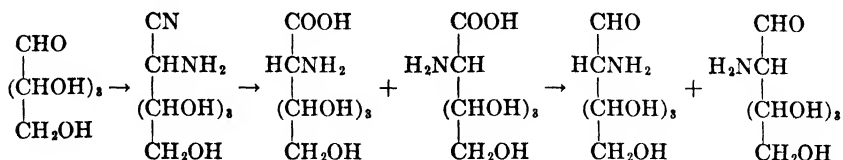
In the second research, 3,5-anhydromonoacetoneoxylose and 5,6-anhydromonoacetoneglucose were heated with the sodium salt of the appropriate alkyl mercaptan. The anhydro ring was thus opened by addition of the mercaptan, which apparently became attached to the terminal carbon.



In each of these investigations it was assumed, without experimental confirmation, that Walden inversion had not occurred, and that the thio compound had the configuration of the original sugar.

Amino Sugars. The amino sugars³ are rather widely distributed in nature, particularly as constituents of muco- and other proteins, and of the polysaccharide, chitin. All the naturally occurring amino sugars are hexoses, and the amino group is invariably on the second carbon atom. Inasmuch as at the present time there is no direct method of establishing the point at which a Walden inversion occurs, the configuration of these compounds cannot be definitely stated. However, on the basis of indirect evidence there seems to be general agreement that chitosamine has the configuration of *d*-glucose, epichitosamine that of *d*-mannose, and chondrosamine that of *d*-galactose. The synthesis of this group of compounds has been effected by adding ammonia and hydrogen cyanide to the appropriate pentose, hydrolyzing the product to the acid,

separating the two epimers, converting to the lactone, and reducing to the aminohexose:

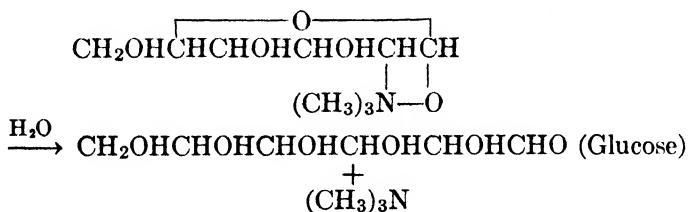


In this way all eight possible 2-amino-*d*-hexonic acids have been prepared, but only four of the possible 2-amino-*d*-hexoses are known.

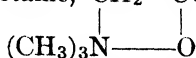
In many respects these amino sugars resemble the ordinary sugars. Benzylidene derivatives have been prepared as well as certain of the pentaacetates, oximes, semicarbazones, and phenylhydrazones. The acetobromo derivative of chitosamine has been obtained and with phenols gives ordinary pyranosides, hydrolyzable by emulsin. With methyl alcohol, however, the glycoside which is secured is abnormal in that the methyl group is extremely resistant to acid hydrolysis and is not hydrolyzed by emulsin.^{9a} Further methylation of the glycoside gives a dimethylaminomethylglycoside, and this on alkaline hydrolysis loses methyl and amino groups and is converted to glucose. This series of reactions has been interpreted by assuming that the original methyl-

glycoside is really the cyclic compound $\text{CH}_2\text{OH} \overbrace{\text{CHCHOHCHOHCHCH}}^{\text{O}}$
 $\text{CH}_3\text{N}-\text{O}$

which on methylation and hydrolysis loses the $\text{N}(\text{CH}_3)_3$ group:



This reaction is of three-fold interest: first, because it has been used as a basis for postulating a structure for the polysaccharide chitin; second, because of its possible relationship to the occurrence, in nature, of betaine, CH_2-CO , and similar compounds; and third, because it

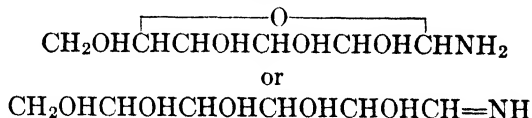


would establish, in the absence of any Walden inversion, the configuration of chitosamine. In this last connection it is interesting to see that precisely the opposite correlation (i.e., to mannose) results from a

^{9a} Irvine and Hynd, *J. Chem. Soc.*, **103**, 41 (1913).

different series of reactions. Thus, if the same aminomethylglycoside used above is converted into the benzylidene derivative, and this then treated with nitrous acid in the presence of sodium nitrite to avoid excess acidity, the methyl and amino groups are simultaneously eliminated and a benzylidenehexose results. Mild acid hydrolysis removes the benzylidene group and mannose is secured. Since glucose resulted in the former reaction it is evident that in one or the other a Walden inversion has taken place, and in the absence of supplementary data no conclusions as to configuration are possible.

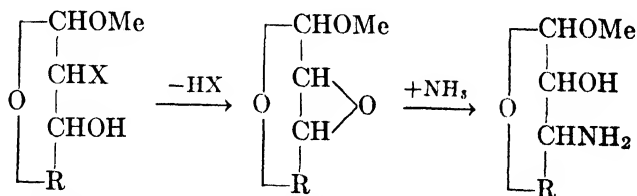
Although the only amino sugars found in nature have the amino group in position two, those in which it is in other positions have been prepared synthetically. Thus 1-aminoglucose (glucosimine) has been obtained from glucose and alcoholic ammonia, and more recently¹⁰ a general synthesis of the 1-aminoaldose derivatives has been reported which involves simply dissolving the aldose in liquid ammonia and evaporating the excess solvent. On the basis of the behavior of the oximes and hydrazones of the aldoses, it may be assumed that these compounds can exist both in ring and open-chain forms:



However, this problem has not been deeply explored. Another 1-amino sugar is the 1-aminofructose which was obtained by Fischer by reducing glucosazone with zinc dust and acetic acid:



In addition to these 1-amino sugars, two hexosamines with the amino group in position three have been prepared synthetically. The first of these is obtained by treatment of 2-bromo-, 2-chloro-, or 2-tosyl- β -methylglucoside with ammonia.^{10a, 10c} In each case the amino group becomes attached to carbon three, presumably through intermediate formation of an anhydro compound:



¹⁰ Muskat, *J. Am. Chem. Soc.*, **56**, 693 (1934).

^{10a} Fischer, Bergmann, and Schotte, *Ber.*, **53**, 516, 539 (1920).

The product was named "methyl epiglucoamine" by Fischer, although this nomenclature is unfortunate in that it indicates a 2-aminohexose, epimeric with glucosamine. This is quite erroneous as was shown by Levene and Meyer, who prepared the osazone and found that the amino group had been retained.^{10b}

It is evident that, in the above reaction, there is a possibility of Walden inversion on either or both of the carbon atoms involved, so that the aminohexose might have any one of four possible configurations. Of the four, Haworth, has tentatively chosen^{10c} that of either altrose or mannose. An interesting peculiarity of this aminohexose is the behavior of its glycoside on attempted acid hydrolysis. Fischer and co-workers considered the glycoside to be non-hydrolyzable, even with rather strong acid, as no reducing sugar was secured. This view was corrected by Levene, who showed that the methyl group was split off in the usual fashion, but that the free sugar underwent spontaneous loss of water, forming a non-reducing anhydro sugar in which the amino group was retained. The structure of this anhydro amino sugar has not been elucidated.

The second of the synthetic 3-aminohexoses was secured by Freudenberg by the action of ammonia on 3-tosyldiacetoneglucose.^{10d} Here the reaction appears to be confined to carbon atom three, and the only doubt concerns the occurrence or non-occurrence of Walden inversion. In the former case the product would be 3-aminoaltrose, and in the latter 3-aminoglucose. Indirect evidence suggests that the altrose configuration is the correct one.

Two 6-aminohexoses have also been synthetically prepared: those of glucose and of galactose. They result upon treatment of the 6-halogeno- or 6-tosylhexose (usually an acetone derivative or the glycoside) with ammonia.^{10e} Since in this series the reaction is confined to a non-asymmetric carbon there is no possibility of Walden inversion and the configuration of these sugars can be stated with certainty.

Brief mention may be made at this point of lactoflavin (p. 1013), which is one of the components of the vitamin B complex. This interesting substance has been shown to be a derivative of 1-amino-*D*-ribitol and not only has it been synthesized but so have several isomeric derivatives. In the flavin the amino group of the sugar forms a part of an isoalloxazin nucleus. It has been found that substitution of *D*- or *L*-arabinose

^{10b} Levene and Meyer, *J. Biol. Chem.*, **55**, 221 (1923).

^{10c} Bodycote, Haworth, and Hirst, *J. Chem. Soc.*, 151 (1934).

^{10d} Freudenberg and Doser, *Ber.*, **58**, 294 (1925); Freudenberg, Burkhart, and Braun, *Ber.*, **59**, 714 (1926).

^{10e} Fischer and Zach, *Ber.*, **44**, 132 (1911); Ohle and v. Vargha, *Ber.*, **61**, 1207 (1928).

for *d*-ribose reduces the biological activity while the *d*-xylo derivative is inactive.

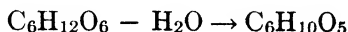
Before leaving the amino sugars it is necessary to mention one of their most interesting reactions, that with nitrous acid. In one of the examples cited above, treatment with nitrous acid produced the usual conversion of an amino to a hydroxyl group, and a hexose resulted. This is not generally true, however; more often there is a simultaneous loss of water and anhydro sugars are formed. These products will be more fully discussed in a later section devoted to the anhydro sugars.

DERIVED SUGARS

From the amino sugars attention may be turned to a group of substances which are important for their chemical as well as for their biological associations. They are derived from the monoses by removal of the elements of water, or an oxygen atom, or both water and an oxygen atom. Their relationships may be indicated as follows:

Product	Formed from Monoses by Elimination of	Ethylenic Linkage in Product
Anhydro sugars.....	H—OH	None
Glycoseens.....	H—OH	One
Glycals.....	HO—OH	One
Desoxy sugars.....	O	None

Anhydro Sugars. The anhydro sugars, as indicated above, may be considered as being derived from the monoses by elimination of the elements of water.



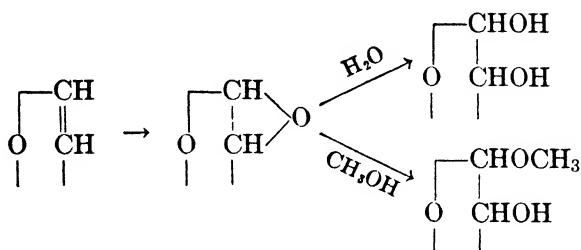
However, in the reaction an internal ether is formed, and this new ring might be, *a priori*, between any two carbon atoms. Actually a great variety of anhydro rings have been reported, covering all the possible forms from ethylene oxide to hexylene oxide.

In the *ethylene oxide* series the 1,2-anhydro derivatives have been given the special name α -glycosans by Pictet^{10/} who prepared them by heating the sugars under reduced pressure to eliminate the elements of water. They were reported by these workers as being reasonably stable, and could in fact be crystallized from methyl alcohol. A reaction which was claimed to prove their structure was the addition of

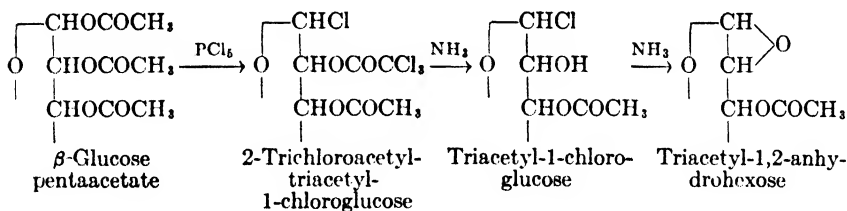
^{10/} Pictet and Castan, *Helv. Chim. Acta*, **3**, 645 (1920); *Compt. rend.* **171**, 243 (1920).

methyl iodide to form substances which were apparently 2-iodomethylglycosides.

Derivatives of this ethylene oxide type are presumably the intermediates in the oxidation of glycols (see below) by peracids. When glucal is treated with moist perbenzoic acid the major product is mannose, whereas with dry perbenzoic acid followed by methyl alcohol the product is largely a methylmannoside. The reactions are presumably the following:



In view of these results, the relative non-reactivity of the α -glycosans described by Pictet is surprising. Brigl,¹¹ however, has synthesized an acetyl derivative in the following manner:



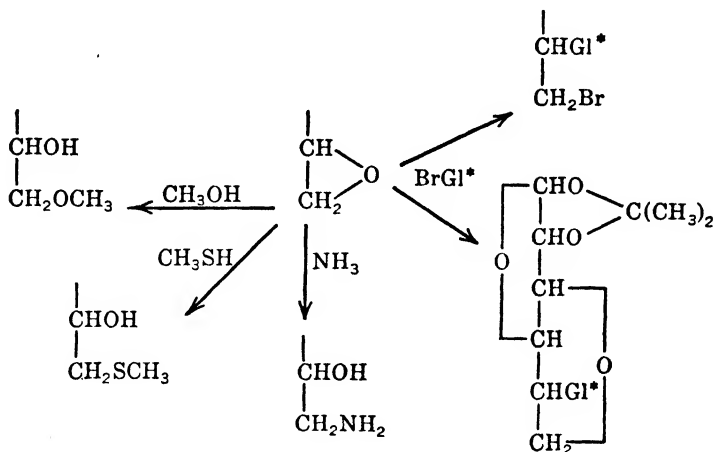
This derivative exhibits the expected reactivity, the anhydro ring being readily opened by various reagents, such as methyl alcohol and acetic anhydride. To explain the difference in reactivity between this substance and Pictet's α -glucosan, it has been assumed that the latter is 1,2-anhydroglucose and that Brigl's compound and the glucal intermediate are 1,2-anhydromannose.

Of the other possible ethylene oxide derivatives, 5,6-anhydromonoacetoneglucose is the best known, being easily prepared from the 6-tosyl derivative by careful treatment with sodium methoxide. The ring is a little more difficult to open than that in the 1,2-anhydro compound (of Brigl), but additions may be effected without much trouble. 6-Methylglucose is formed with sodium methoxide, 6-aminoglucose

¹¹ Brigl, *Z. physiol. Chem.*, **116**, 1 (1921).

with ammonia, 6-thiomethylglucose with sodium methyl mercaptan (CH_3SNa), and apparently two substances with acetobromoglucose.

These reactions may be indicated as follows:

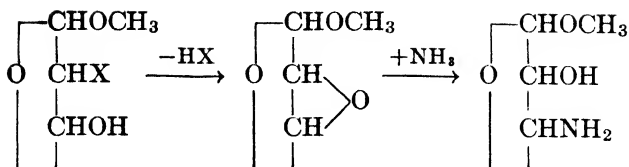


A fact of considerable theoretical importance is that the 5,6-anhydroglucose derivative, on alkaline hydrolysis,¹² gives a mixture of glucose and idose, but on acid hydrolysis only glucose. In this connection may be mentioned the interesting observations of Levene and Walti, who found that acid hydrolysis of optically active propylene oxide gave an excess of one optical isomer, while alkaline hydrolysis gave an excess of the opposite form.

One further ethylene oxide anhydro sugar is a 4,6-dimethyl-2,3-anhydro- α -methylhexoside, prepared by treatment of 2,3-ditosyl-4,6-dimethyl- α -methylglucoside with alkali. A simultaneous product of the reaction is a 4,6-dimethyl- α -methylhexoside. Since two asymmetric carbon atoms are involved, and since Walden inversion might occur on either or both of them, four configurations require consideration for each product. Certain of these possibilities were excluded experimentally, and it was concluded that the anhydrohexoside was probably either an allose or a mannose derivative, while the hexoside was probably an altrose derivative. As mentioned previously a 2,3-anhydrohexose similar to the above is presumably formed as an intermediate by the action of ammonia on 2-bromo, 2-chloro-, or 2-tosyl- β -methylglucoside in the preparation of epiglucoamine. However, it has not as yet been isolated.

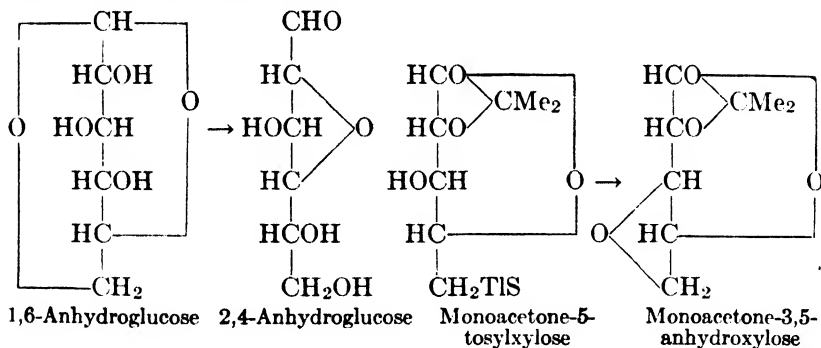
* "Gl" is used here as a abbreviation for the tetraacetylglucose radical, and "BrGl" for acetobromoglucose.

¹² Ohle and von Varga, *Ber.*, **62**, 2425 (1929).



The conversion of glucose to galactose, suggested by Robinson (p. 1480) as perhaps occurring enzymically through an intermediate ester, has actually been achieved by a non-enzymic reaction. Treatment of 2,3-dibenzoyl-4-tosyl-6-triphenylmethylglucose with alkali gives a 3,4-anhydro derivative, and this on treatment with hydrogen chloride gives a mixture of *d*-glucose and *d*-galactose.^{12a} In similar fashion monoacetone-3-tosylfructopyranose can be converted to a 3,4-anhydro derivative which with sodium methoxide gives *d*-sorbose, or with sodium hydroxide, a mixture of *d*-sorbose and *d*-fructose.

Of the *propylene oxide* anhydro sugars only two have been described. One of these is 2,4-anhydroglucose, formed by a complex reaction on treatment of 1,6-anhydroglucose with concentrated hydrochloric acid, and the other is monoacetone-3,5-anhydroxylose, formed from monoacetone-5-tosylxylose with sodium methoxide.



In each of these the ring is relatively unstable; the first can be hydrolyzed with dilute acid to glucose, while the second adds sodium methoxide or sodium methyl mercaptan to form 5-methyl- and 5-thiomethylmonoacetonylose, respectively.

The most stable anhydro rings are the *butylene oxide* type, the 2,5- and the 3,6-anhydro in the hexose series. As previously mentioned, these result on treatment with nitrous acid of the 2- and 5-amino hexoses, elimination of water being spontaneous. It has been proved by Levene that the substance thus formed from epichitosamine is 2,5-anhydroglucose whereas that from chitosamine is 2,5-anhydromannose. This proof

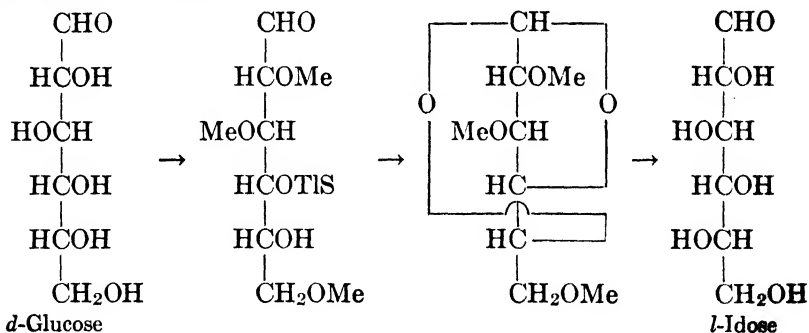
^{12a} Oldham and Robertson, *J. Chem. Soc.*, 685 (1935).

consists in an experimental determination of configuration, entirely analogous to that used for the monoses themselves, and involves no considerations of Walden inversion.

In addition to this method of formation, the 3,6-anhydro compounds have also been secured by removing the elements of hydrogen bromide from the methyl-6-bromoglycosides by treatment with alkali. In the case of glucose the product thus prepared is not identical with that obtained by deaminizing epiglucoamine, but appears rather to be epimeric.

One of the 3,6-anhydroglucoses has been hydrolyzed by dilute acids, glucose being regenerated, but the 2,5-anhydro rings are so stable that their hydrolysis has not as yet been accomplished.

A substance which may be considered as being both a *butylene* and an *amylen*e oxide is the extremely interesting compound prepared by Hess and Neumann.¹³ They treated 2,3,6-trimethyl-4-tosylglucose with alkali, the tosyl group was eliminated, and a 1,4-anhydro compound resulted. Since the original 1,5-ring is retained, the product is simultaneously a furanose and a pyranose. Interestingly, on treatment with hydrobromic acid, the rings are opened, the methyl groups are split off, and there is a simultaneous Walden inversion on the fifth carbon, giving rise to *l*-idose.

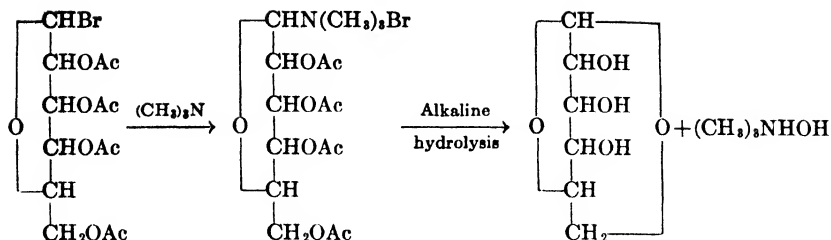


The best known of the 1,6-anhydrohexoses is the levoglucosan^{13a} of Pictet, prepared by destructive distillation of starch or other polysaccharides under reduced pressure. The compounds belonging to this series may also be prepared synthetically¹⁴ by adding trimethylamine to the acetobromo derivative and then hydrolyzing with alkali whereby the acetyl groups and the trimethylammonium group are split off and the 1,6-anhydro derivative is secured.

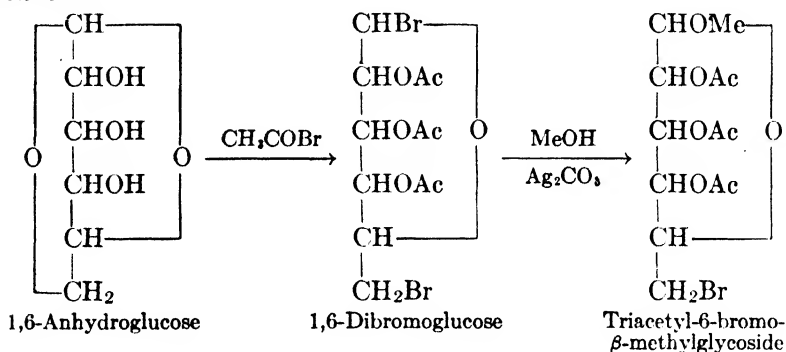
¹³ Hess and Neumann, *Ber.*, **68**, 1360 (1935).

^{13a} Pictet and Sarasin, *Helv. Chim. Acta*, **1**, 87 (1918); Pictet and Cramer, *ibid.*, **3**, 640 (1920). See, also, Tanret, *Bull. soc. chim.*, [3] **11**, 949 (1894) and Vongerichten and Müller, *Ber.*, **39**, 241 (1906).

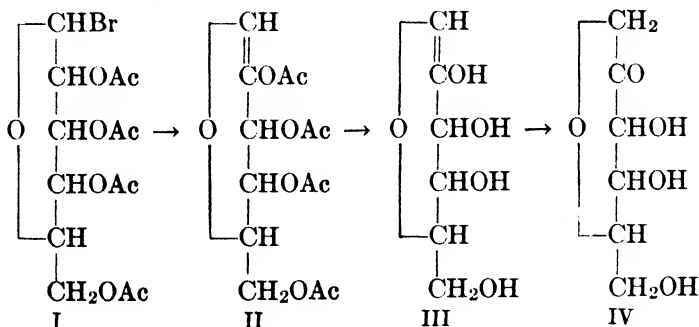
¹⁴ Mischeel, *Ber.*, **62**, 687 (1929); Karrer and Smirnoff, *Helv. Chim. Acta*, **4**, 817 (1921).



Opening of the 1,6-ring may be effected by various reagents, for example by acetyl bromide, producing 1,6-dibromotriacetyl-glucose, which in turn may be converted to the glycoside for use in various syntheses.

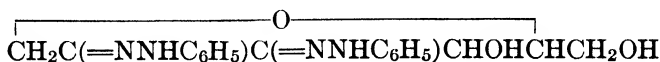


Glycoseens. The glycoseens differ from the anhydro sugars in that a double bond is formed during the elimination of the elements of water from the aldoses. The two best-known types are those with the double bond between carbons one and two, or between carbons five and six. The *1,2-glycoseens* are prepared¹⁵ by reacting the acetobromo sugar (I) with diethylamine, whereby hydrogen bromide is eliminated and tetraacetyl-1,2-glycoseen (II) is formed.



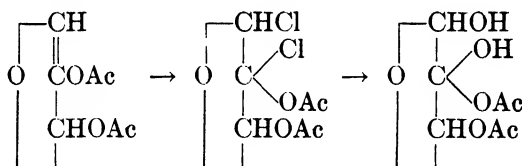
¹⁵ Maurer and Mahn, *Ber.*, **60**, 1316 (1927); Maurer, *Ber.*, **62**, 332 (1929).

This will be seen to be the acetate of an enolized 1,5-anhydro-2-ketohexose (III) and might be expected to revert to the keto form (IV) on deacetylation. Actually, on attempted deacetylation no definite substance has been secured, but reacetylation of the product thus obtained does not give the original acetylglucoseen. With phenylhydrazine, however, the product is apparently

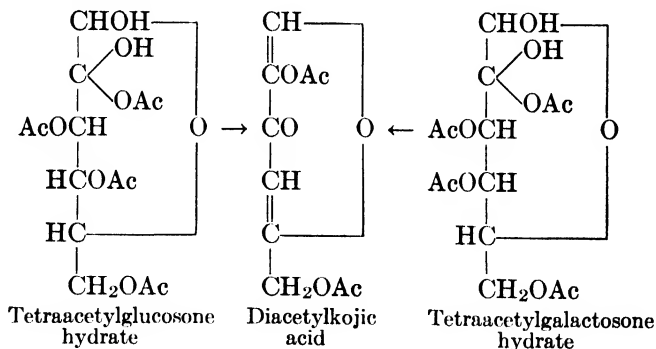


which is the "osazone" of IV.^{15a} It is of interest that treatment of tetraacetyl-1,2-glucoseen with excess alkali, and back titration with acid, as in an acetyl determination, uses five and not four equivalents of alkali.

Tetraacetyl-1,2-glucoseen reacts with chlorine, giving a crystalline compound which decomposes spontaneously. However, the chlorine may be removed by immediate hydrolysis with moist silver carbonate and there then results the acetate of glucosone hydrate.¹⁶



This acetate is readily converted with acetic anhydride and pyridine to diacetylkojic acid. Owing to loss of configuration this same product may be similarly prepared from tetraacetyl-1,2-galactoseen (through galactosone hydrate).



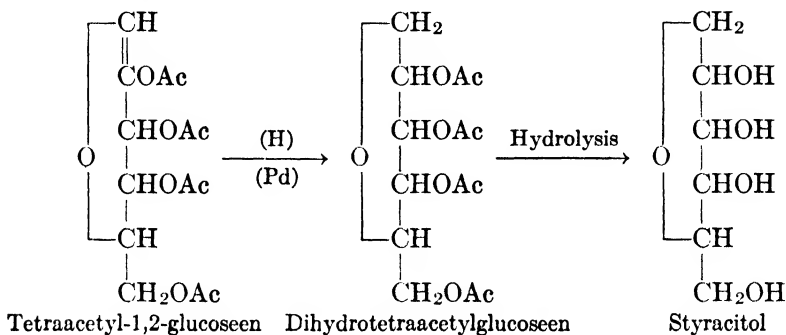
Kojic acid is of biological interest as it is formed in appreciable amounts

^{15a} Bergmann and Zervas, *Ber.*, **64**, 1434, 2032 (1931).

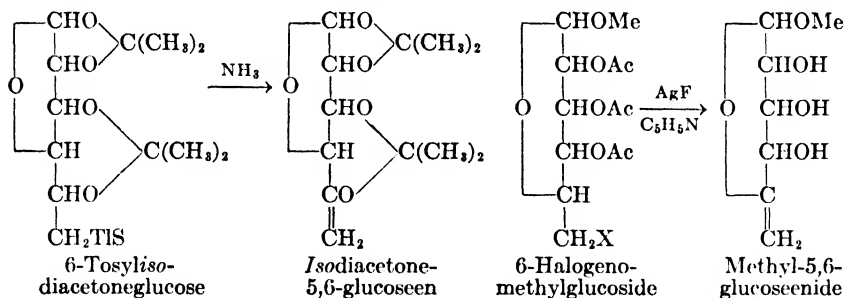
¹⁶ Maurer, *Ber.*, **63**, 25 (1930); Maurer and Müller, *Ber.*, **63**, 2069 (1930).

by the action of many molds not only upon hexoses, but also upon pentoses, trioses, glycerol, and other substances.

Tetraacetyl-1,2-glucoseen has been used by Zervas^{16a} to establish the structure of styracitol. Hydrogenation of the glucoseen, using a palladium catalyst, and hydrolysis of the resultant tetraacetyl derivative produced styracitol which was therefore stated to be 1,5-anhydro-sorbitol. It should be noted, however, that the configuration of carbon atom two has not been established by this synthesis.



Analogous to 1,2-glucoseen is 5,6-glucoseen. This is most easily prepared by reacting the 6-bromo or 6-iodo compound with silver fluoride in pyridine solution although it is also one of the products of the reaction of 6-tosylisodiacetoneglucose and alcoholic ammonia.^{16b}

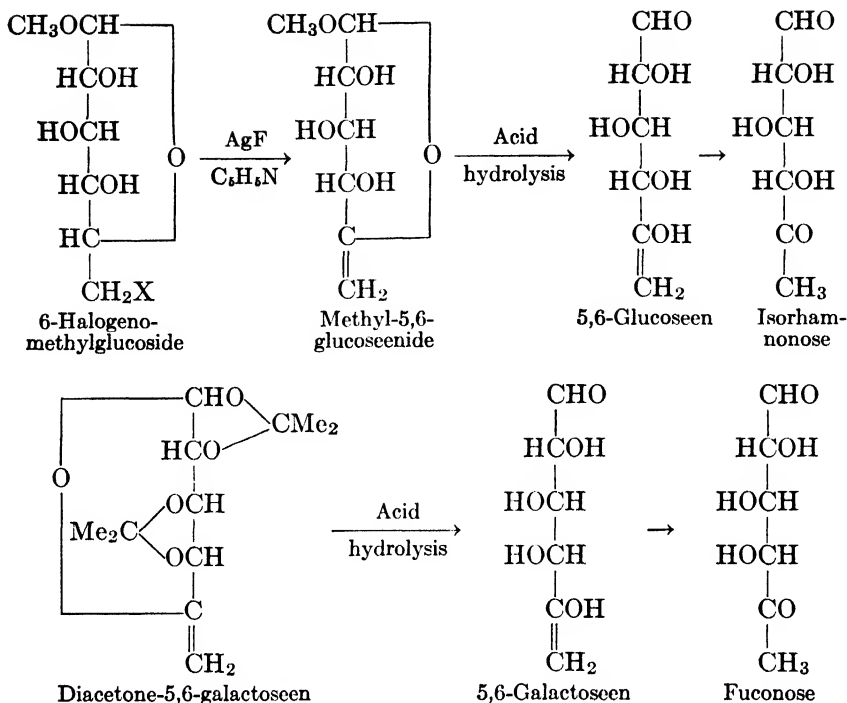


Like the 1,2-glucoseen it may be considered to be an enol, stabilized by the internal ether ring. Upon acid hydrolysis the ring is destroyed and the product ketonizes, giving a 5-keto-6-desoxyhexose, isorhamnonose. Similarly, diacetone-5,6-galactoseen on acid hydrolysis gives the isomeric fuconose.¹⁷

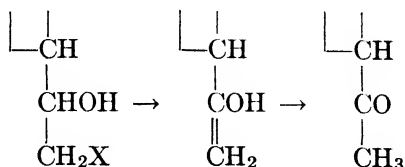
^{16a} Zervas, *Ber.*, **63**, 1689 (1930).

^{16b} Helferich and Himmen, *Ber.*, **61**, 1825 (1928); Ohle and v. Vargha, *Ber.*, **62**, 2425 (1929).

¹⁷ Helferich and Himmen, *Ber.*, **62**, 2136 (1929); Ohle and Deplanque, *Ber.*, **66**, 12 (1933).



In addition to the intrinsic interest of a reaction of this type, a further interest attaches to it in connection with the alkaline rearrangements which will be considered later and which involve an internal oxidation and reduction. It was also recently suggested¹⁸ that this reaction might be used to distinguish between furanohexosides and pyranohexosides as the former should give immediately a keto compound

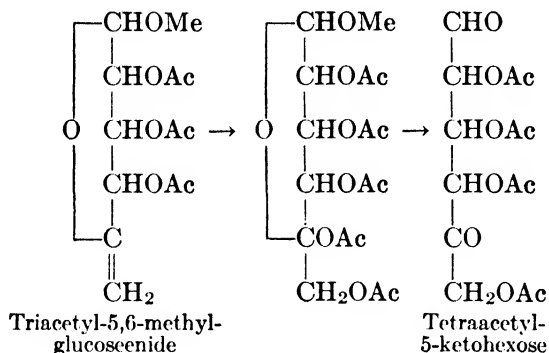


whereas the latter could do so only after hydrolysis, as indicated above. Experimentally, however, it was found that appropriate derivatives of both mannitol and glucose^{18a} failed to react with silver fluoride in pyridine, and no further evidence along this line has been obtained.

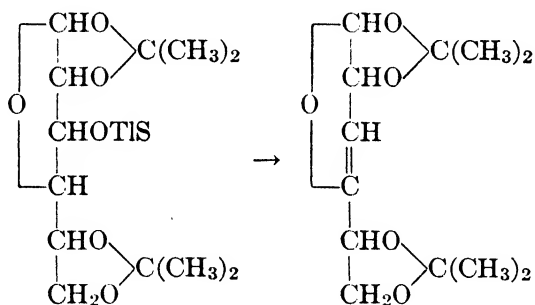
¹⁸ Müller, *Ber.*, **65**, 1051 (1932).

^{18a} Helferich and Lang, *J. prakt. Chem.*, **132**, 321 (1932).

A final reaction of 5,6-glucoseen which may be noted is oxidation with lead tetraacetate^{18b} to a derivative which hydrolyzes in water and gives a 5-ketohexose:



In addition to the 1,2- and 5,6-glucoseens, one example of the 3,4-*glycoseens* has been described. This was formed as a by-product in the reaction of 3-tosyldiacetoneglucose with hydrazine. Although its structure has not been confirmed, it appears to be diacetone-3,4-glucoseen,

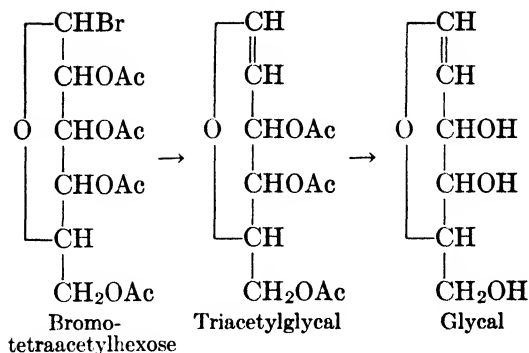


thus providing an additional example of this interesting group of substances.

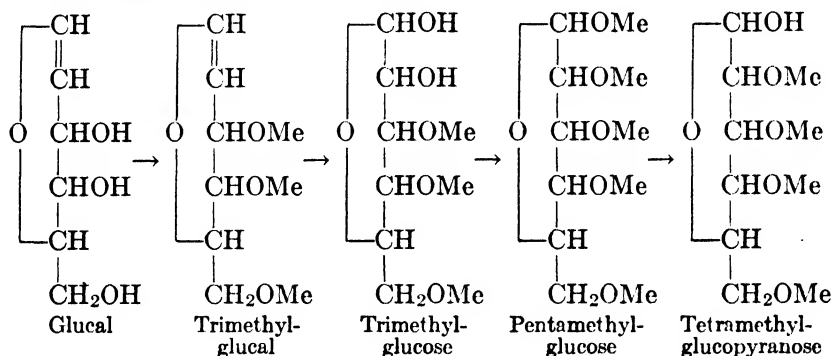
Glycals. The glycals are unsaturated derivatives, formed by reduction of the acetobromo compounds with zinc in acetic acid, followed by hydrolysis of the acetyl groups.¹⁹ They differ from the parent aldose in having lost an oxygen atom and the elements of water, while a double bond has appeared.

^{18b} Helferich and Bigelow, *Z. physiol. Chem.*, **200**, 263 (1931).

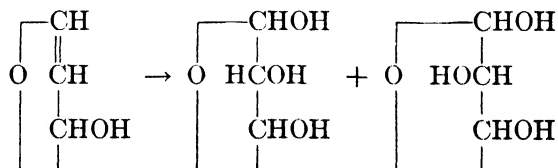
¹⁹ Fischer, *Ber.*, **47**, 196 (1914); Bergmann and Schotte, *Ber.*, **54**, 446 (1921); Bergmann and Freudenberg, *Ber.*, **62**, 2783 (1929).



With glucose, it has been shown that this series of changes is unaccompanied by a shift in ring,²⁰ for methylation of glucal, oxidation with perbenzoic acid, further methylation, and hydrolysis give ordinary tetramethylglucopyranose (p. 1423).



The oxidation of the glycal with perbenzoic acid is one of the most interesting reactions of this group of compounds. Apparently a mixture of the two epimers is invariably formed:



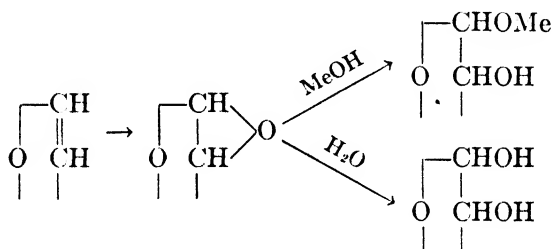
but the proportions of the two may vary widely,^{20a} and the conditioning factors are not well understood. Thus with glucal and rhamnal, the products are almost exclusively mannose and rhamnose, whereas with

²⁰ Hirst and Woolvin, *J. Chem. Soc.*, 1131 (1931).

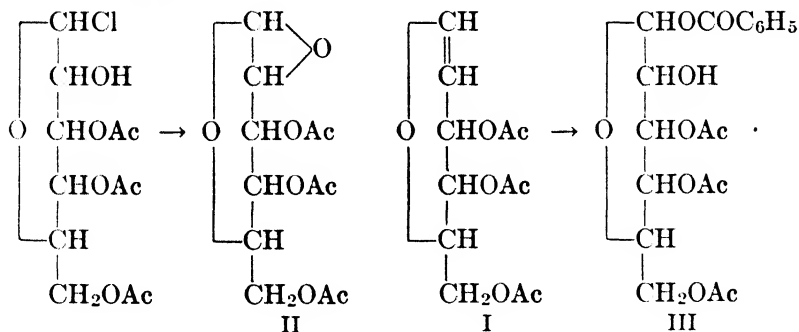
^{20a} Bergmann and Schotte, *Ber.*, **54**, 440 (1921); Tanaka, *Bull. Chem. Soc. Japan*, **5**, 214 (1930); Levene and Raymond, *J. Biol. Chem.*, **88**, 513 (1930); Hirst and Woolvin, *J. Chem. Soc.*, 1131 (1931); Levene and Tipson, *J. Biol. Chem.*, **93**, 631 (1931).

galactal, although talose predominates, much galactose is formed. Moreover, substitution of glugal in position three may change the proportions enormously, for the glucose derivative predominates on oxidation of triacetylglugal and 3-methylglugal, and trimethylglucose is the principal or perhaps only product from trimethylglugal.

The mechanism of this oxidation with perbenzoic acid has not been fully established. As mentioned, the oxidation of glugal in the presence of moisture leads to the production of mannose. In the absence of moisture, however, if the intermediate product is treated with methyl alcohol, α -methylmannoside is formed, and in similar fashion α -methylrhamnoside is formed from rhamnal. This indicates the intermediate occurrence of a 1,2-anhydro sugar, but isolation of a compound of this nature from the reaction mixture has not as yet been achieved.



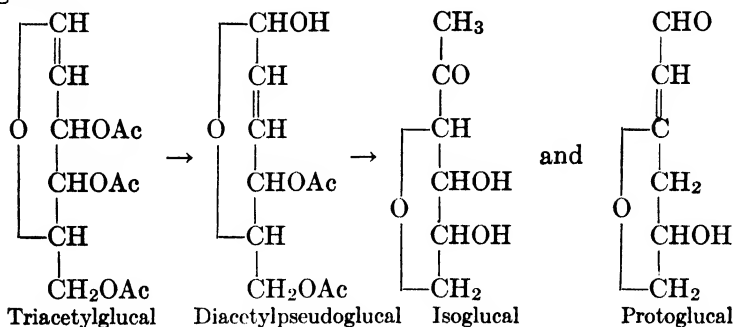
It might be expected that triacetylglugal (I) with perbenzoic acid would give a compound identical with that (II) prepared by Brigl (see 1,2-anhydro sugars, p. 1489).



Interestingly, however, the major substance which is isolated from this oxidation is 1-benzoyl-3,4,6-triacetylglucose (III), although other glucose and mannose derivatives are simultaneously formed.

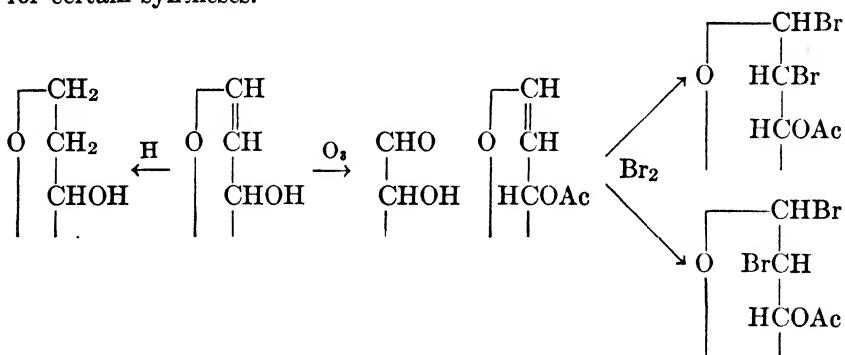
Other reactions of the glycals have to do with their isomerization. Thus when triacetylglugal is boiled with water, diacetylpsudoglugal is formed, and this, on hydrolysis with barium hydroxide solution, gives

by an elaborate rearrangement isoglucal, and in lesser amount, protoglucal.²¹

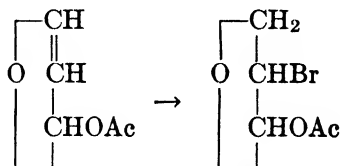


Protoplucal reduces Fehling's solution, and its presence in traces is probably responsible for the original erroneous report that glucal is a reducing substance.

The glycols may be oxidized by ozone to give the corresponding aldose of one less carbon atom, which incidentally proves the position of the double bond, or they may be hydrogenated to the hydroglycols. Halogens may be added to triacetylglucal to give a mixture of two epimeric acetohalogeno-2-halogenohexoses which have proved useful for certain syntheses.



The addition product with hydrogen bromide appears to have the bromine in the 2-position instead of in the 1-position.²²



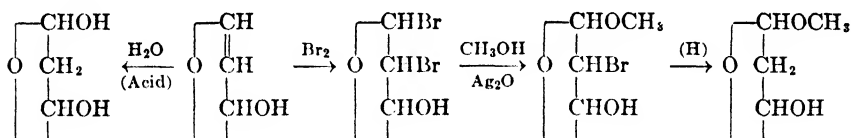
²¹ Bergmann and Freudenberg, *Ber.*, **64**, 158 (1931).

²² Fischer, Bergmann, and Schotte, *Ber.*, **53**, 517 (1920).

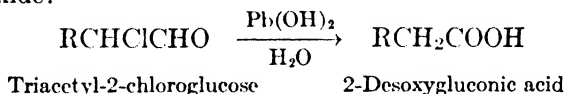
This is unfortunate as otherwise there would be easily available the acetobromo derivatives of the 2-desoxy sugars, which should be useful for synthetic work in this series.

Desoxy Sugars. Although originally of only chemical interest, desoxy sugars (p. 1451) from several natural sources have been described. Several of the cardiac glycosides contain desoxy sugars as a component (digitoxose, cymarose, sarmentose), and 2-desoxyribose (-arabinose) has been shown to be the sugar of thymus nucleic acid. It is of interest that cymarose (p. 1454) is a methyl ether (of digitoxose) and is on this account quite unusual among the naturally occurring sugars.

The desoxy sugars owe their name to the fact that one or more CHOH groups have been deprived of an oxygen atom and converted into a CH₂. The synthetic preparation of the 2-desoxy sugars has been achieved from the glycals in two ways. In one of these the 2-halogeno aldoses described above are reduced, and in the other water is added directly to the glycal, usually with sulfuric acid as catalyst, as indicated:²³



An interesting method for preparing the 2-desoxygluconic acids has recently appeared in which an intramolecular oxidation and reduction results when 2-chloroglucose (triacetyl- or trimethyl-) is heated with lead hydroxide:²⁴

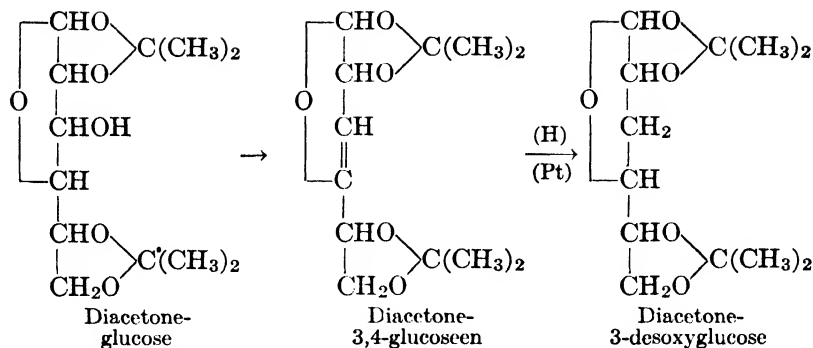


A characteristic of the 2-desoxy sugars is their great reactivity. The pyranosides in this series, for example, are sometimes formed or hydrolyzed as rapidly as are the furanosides of the ordinary sugars. Only a single 1-bromo derivative in this series has been thus far prepared. The ready formation of levulinic acid from the 2-desoxypentoses on treatment with acid will be discussed later (p. 1509).

A single 3-desoxy sugar has been described as resulting from the catalytic reduction of diacetone-3,4-glucoseen but these structures have not been well authenticated.

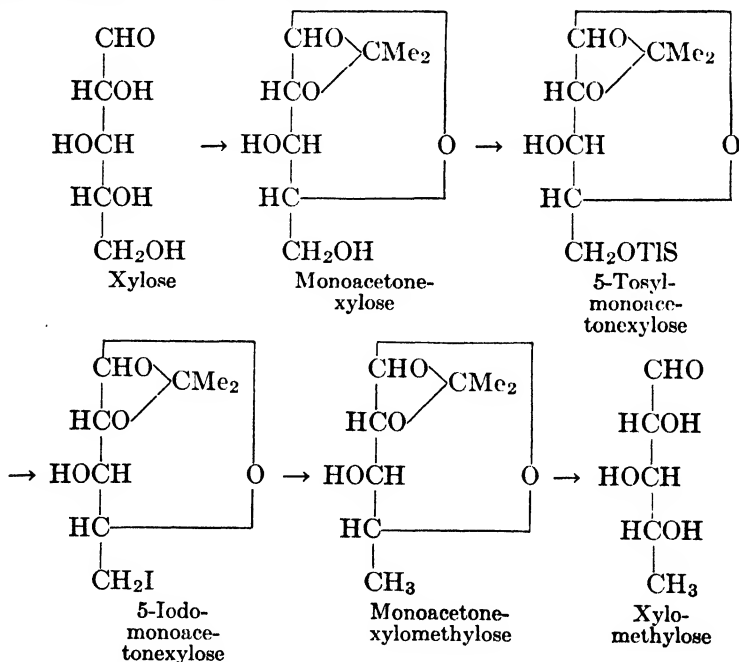
²³ Bergmann, Schotte, and Leschinsky, *Ber.*, **56**, 1052 (1923); Gehrke and Aichner, *Ber.*, **60**, 918 (1927); Levene and Mori, *J. Biol. Chem.*, **83**, 803 (1929); Levene, Mikeska, and Mori, *ibid.*, **85**, 785 (1930).

²⁴ Danilov and Gakhokidze, *J. Gen. Chem. (U.S.S.R.)*, **6**, 704 (1936).



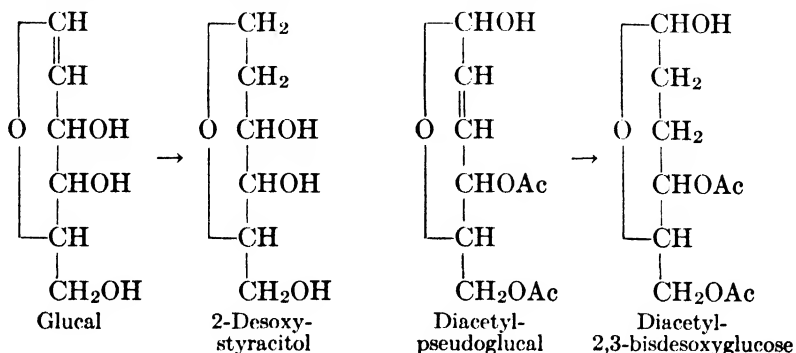
Very widely distributed in nature are a further series of sugars, the methyloses, in which the terminal group is the desoxy (p. 1450). Although much work has been done in this series, it seems sufficient to indicate the synthetic method of their preparation, which is that of reduction of a halogen attached to the terminal carbon. This is conveniently the iodide, introduced by replacing the tosyl group, although other halogens have been similarly reduced.

As a typical example of a synthesis in this series the preparation of *d*-xylomethylose ^{24a} may be cited:



^{24a} Levene and Compton, *J. Biol. Chem.*, **111**, 325 (1935).

It is possible, of course, to have more than one carbon as a desoxy group, and the hydrogenation products of the glycols afford examples of this type of compound. Thus dihydroglucal is really 2-desoxystyracitol, and dihydropseudoglucal is 2,3-bisdesoxyglucose.



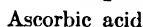
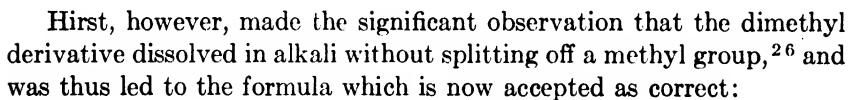
The naturally occurring digitoxose is, in fact, a bisdesoxy sugar, 2-desoxyallomethylose (or 2,6-bisdesoxyallose) (p. 1451).

Ascorbic Acid—Vitamin C. The rapid and successful solution of the ascorbic acid problem, both as to structure and synthesis, constitutes one of the major achievements in the field of sugar chemistry, and on this account is deserving of special consideration. Inclusion at this point is justified by the fact that the vitamin is an unsaturated sugar derivative, and because the problems connected with it give valuable information in the field of these unsaturated derivatives.

Szent-Györgyi had isolated from a number of vegetable and plant sources, as well as from suprarenal cortex, a "hexuronic acid," which he considered to be associated with respiratory processes. He had, moreover, pointed out that the distribution of this substance paralleled that of vitamin C. Although Zilva claimed that there was no constant relationship between the antiscorbutic activity and the reducing property which was a characteristic of Szent-Györgyi's acid, this was disputed some years later by Tillmans and co-workers who found that such a relationship did in fact exist. Szent-Györgyi and his co-workers next demonstrated for their crystalline material a definite antiscorbutic activity, and King with his collaborators independently and simultaneously described a crystalline vitamin C preparation which had all the physical and chemical properties of the "hexuronic acid."²⁵ After further investigation it was at length agreed that the two substances were identical. Szent-Györgyi was finally able to secure relatively large

²⁵ For review of literature see "Annual Review of Biochemistry," Stanford University Press, Stanford University, Calif. (1934), Vol. II, chapter on vitamins.

"Hexuronic acid," or ascorbic acid as it was soon called, behaves chemically like an unsaturated monobasic acid. It has the empirical formula $C_6H_8O_6$ and contains one double bond. It gives a dimethyl derivative with diazomethane, which is specific for methylation of acidic hydrogens. These facts are all adequately explained on the basis of the earlier formulas, given below, in which one of the acidic hydrogens is that of the carboxyl group and the other is that of the tautomeric hydroxyl:

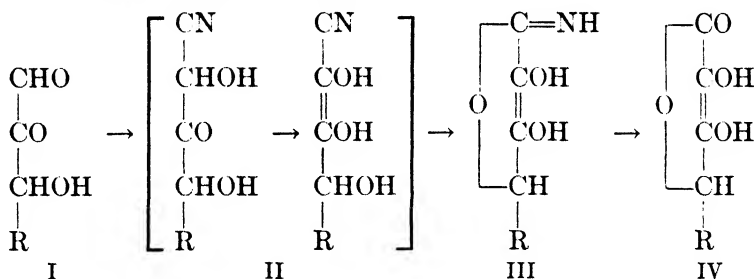


The configuration was deduced from that of the oxidation product of ascorbic acid which was shown to be 2,3-diketo-*l*-gulonic acid, as well as from the fact that ozonization of ascorbic acid and of its methyl deriva-

²⁶ Hirst, *Chemistry & Industry*, **52**, 221 (1933).

tives gave *l*-threonic acid and its methyl ethers. On the basis of this formula, ascorbic "acid" is a highly stable *lactone*, which owes its acidic properties entirely to an enolized keto group. It is of interest that, whereas ozonization of tetramethylascorbic acid, prepared by further methylation of crude dimethylascorbic acid, gives a mixture of 3,4-dimethyl-*l*-threonic acid and the epimeric 3,4-dimethyl-*l*-erythronic acid, if the dimethyl derivative is first isolated in crystalline form and then further methylated and ozonized, only the threonic derivative is secured. This is apparently due to further tautomerizations involving the fourth carbon atom.

That the formula above was in fact correct was soon confirmed by a series of brilliant syntheses, of which the first was that of Reichstein, Grüssner, and Oppenauer.²⁷ These authors started with the osone of *d*-xylose, and by addition of hydrogen cyanide, followed by hydrolysis, effected the synthesis of the enantiomorphic *d*-ascorbic acid. This method was then utilized by Haworth and co-workers^{27a} who started with *l*-xylose and with somewhat modified procedure effected the synthesis of the naturally occurring *l*-ascorbic acid. Further studies on this method have revealed that the mechanism is apparently rather complex, the changes probably being as follows:



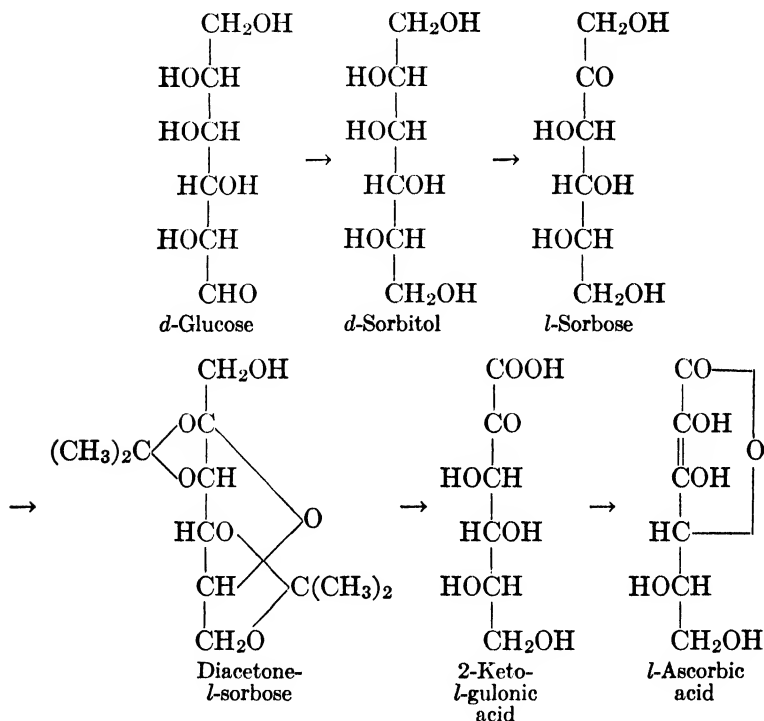
The first intermediate isolated is the crystalline cyclic imino compound (III), and this, on treatment with acid, passes smoothly into the ascorbic acid (IV). This type of synthesis is general, and a number of isomeric "ascorbic acids" have been prepared in this manner.

The second important method of synthesis of ascorbic acid is also due to Reichstein,²⁸ who found that *l*-sorbose (prepared from *d*-sorbitol by the action of *Acetobacter xylinum*; and the *d*-sorbitol from *d*-glucose by catalytic reduction) forms a 2,3,4,6-diacetone derivative which on

²⁷ Reichstein, Grüssner, and Oppenauer, *Helv. Chim. Acta*, **16**, 561, 1019 (1933); **17**, 510 (1934). See, also, Haworth *et al.*, *J. Chem. Soc.*, 1419 (1933); 62, 1192 (1934).
^{27a} Ault *et al.*, *J. Chem. Soc.*, 1419 (1933).

²⁸ Reichstein and Grüssner, *Helv. Chim. Acta*, **17**, 311 (1934).

alkaline oxidation yields diacetone-2-keto-*l*-gulonic acid. Removal of the acetone groups by acid hydrolysis gives the free acid, and this on heating with water is transformed into ascorbic acid. A somewhat better preparation consists in converting the free acid into its methyl ester and heating this with sodium methoxide in methyl alcohol whereby the sodium salt of *l*-ascorbic acid is secured.



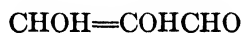
The 2-keto-*l*-gulonic acid, which is an intermediate in this synthesis, was obtained more easily by Haworth,^{28a} who has found that direct oxidation of ketoses with nitric acid leads to preferential oxidation of the primary alcoholic group adjacent to the keto group. In this manner ascorbic acid was easily prepared from *l*-sorbose, and *d*-araboascorbic acid (the nomenclature refers the ascorbic acid to the parent aldose of one less carbon) from *d*-fructose.

A series of synthetic analogs has been obtained by these methods and their physiological activity has been examined. Those prepared are: the only possible four-carbon analog, one of the two possible five-carbon forms, all four of the possible six-carbon acids, three of the eight possible

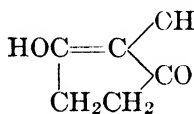
^{28a} Haworth, *Nature*, **134**, 724 (1934); *Brit. Assoc. Advancement Sci. Rept.*, 295 (1934).

seven-carbon acids, and the ascorbic acid from *l*-rhamnose. Of these, the synthetic *l*-ascorbic acid has the same activity as the natural substance, as has its primary oxidation product; *l*-rhamnoascorbic acid has about one-fifth as much activity, *d*-araboascorbic acid has a slight activity, and none of the others may be regarded as possessing any antiscorbutic activity. It is of some interest that the imino compound (III), though closely related to ascorbic acid chemically, is without activity. The generalization, made simultaneously by Reichstein and by Haworth,²⁹ is that for antiscorbutic activity the fourth carbon atom must be of the *d*-series.

Of further importance in this fascinating field are the interesting substances reductone and reductic acid.³⁰



Reductone



Reductic acid

The first of these is the enol of hydroxymethylglyoxal, formed by action of alkali on various carbohydrates; the second is formed by action of dilute sulfuric acid at high temperatures. Both are characterized by the same system of enediols as that in ascorbic acid, and like ascorbic acid both reduce the characterizing indicator, dichlorophenolindophenol. With additional knowledge it may be found that substances of this nature are of great importance in the chemical as well as in the biological degradations and transformations of the sugars.

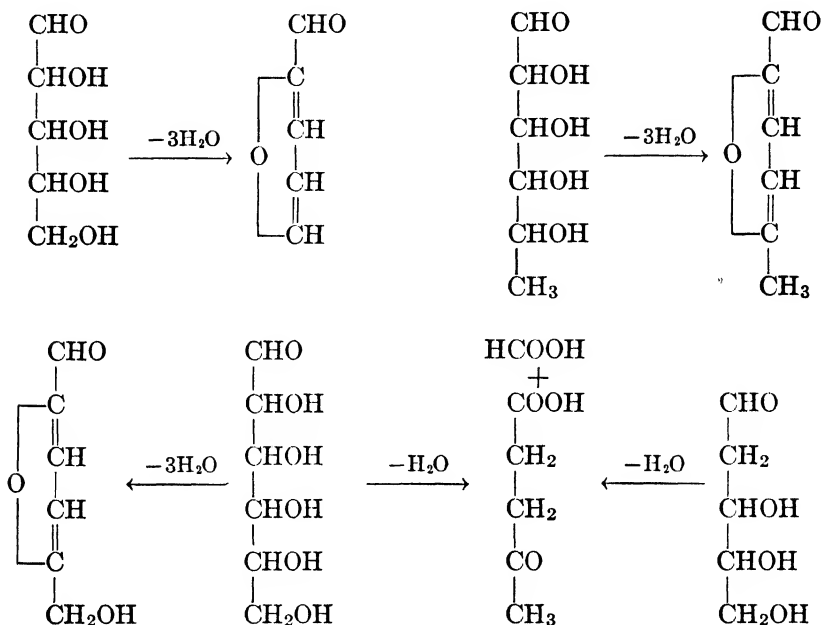
ISOMERIZATIONS AND DEGRADATIONS

Acid Rearrangements. The mechanisms of the reactions whereby the sugars are rearranged or are broken down into smaller fragments is of the greatest interest both chemically and biologically. It has, however, been the subject of so much research that only a brief outline of the conclusions is possible here. The simplest types of changes are effected by *acid* treatment, and the effect ranges from almost nothing with weak acids, to complex changes, eventuating in the formation of humic substances, with hot concentrated acids. Between these extremes lie simple conversions, produced by acids of intermediate concentration, such as the production of furfural from pentoses and the analogous pro-

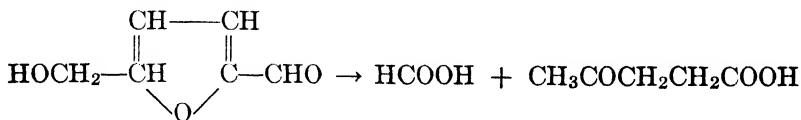
²⁹ Reichstein, *Nature*, **134**, 724 (1934); Haworth, *ibid.*, and *Brit. Assoc. Advancement Sci. Rept.*, 295 (1934).

³⁰ Norrish and Griffiths, *J. Chem. Soc.*, 2837 (1928); von Euler and Martius, *Svensk Kem. Tid.*, **45**, 73 (1933); Reichstein and Oppenauer, *Helv. Chim. Acta*, **16**, 988 (1933).

duction of methylfurfural from methylpentoses, and hydroxymethylfurfural and levulinic acid from hexoses:

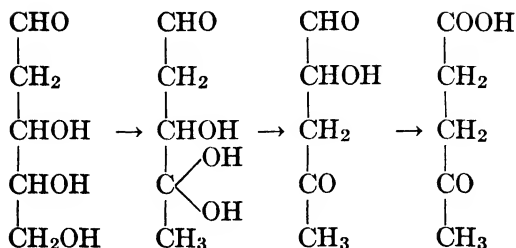


It will be observed that, with the exception of levulinic acid, all the products may be considered as being formed in identical fashion by abstraction of three molecules of water. Levulinic acid results on similar treatment of 2-desoxypentoses as well as of hexoses, and this fact was for a long time responsible for the belief that the sugar of the thymus nucleic acids was a hexose. No very good evidence has been presented to account for the formation of levulinic acid, although it has been shown to result on acid treatment of hydroxymethylfurfural.³¹



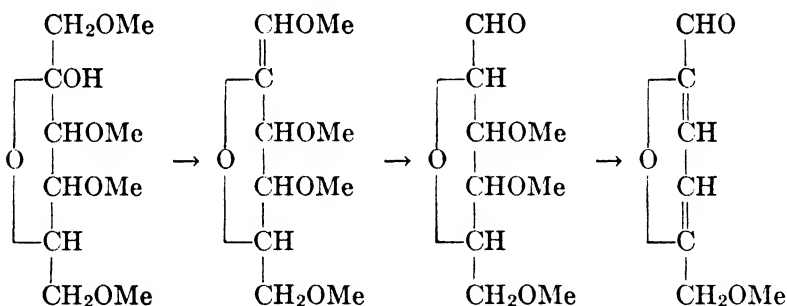
Levene and Mori have indicated the formation from 2-desoxypentoses in the following way,

³¹ Pummerer, Guyot, and Birkofer, *Ber.*, **68**, 480 (1935).



without attempting to substantiate this mechanism.

In connection with the formation of furfural, several observations may be mentioned. The first of these is the fact that the fully methylated pentoses, whether furanose or pyranose, give furfural on treatment with strong acid.^{31a} The yields are in general as high as those from the free pentoses and are sometimes higher. Moreover they appear to be independent of the furanose or pyranose ring form of the methylated sugar. Of interest in the same connection is the formation of methoxymethylfurfural from tetramethylfructofuranose. For this rearrangement Haworth has suggested the following mechanism, which assumes the enol as the first step:

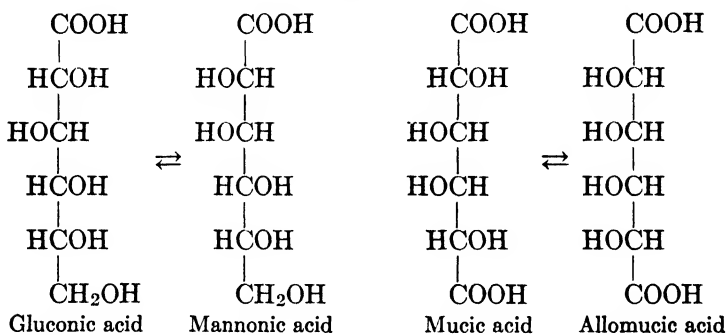


Alkaline Rearrangements. Turning from the action of acids on sugars to that of *alkalies*, a much more complicated problem is encountered. For a logical treatment of the subject the effect of alkalies might be divided into, first, those changes which involve rearrangement without splitting, and, second, the changes involving scission of the molecule into smaller fragments. The first of these classes would include as its simplest case the problem of mutarotation or Walden inversion on the aldehydic carbon atom (which has been discussed, p. 1413), then epimerization or Walden inversion on the carbon adjacent to the aldehydic carbon, next the progressive wandering of the reducing group

^{31a} Bott and Hirst, *J. Chem. Soc.*, 2621 (1932).

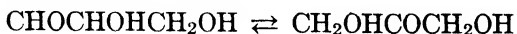
along the carbon chain, and finally such complicated rearrangements as those involved in saccharinic acid formation where branched-chain acids result. In the second of the above classes would be included the formation of aldehydic substances such as formaldehyde, acetaldehyde, and methylglyoxal, acids such as formic, acetic, lactic, and dihydroxybutyric, and finally reduced scission products such as alcohol. Unfortunately, however, in practice it becomes almost impossible to segregate these problems for separate discussion as each type of reaction is intimately concerned with each of the others, and deviation from such logical presentation becomes almost unavoidable.

In the study of epimerization, complex side reactions may be avoided by working with the sugar acids. By heating them with aqueous pyridine, apparently only the epimers are formed and, moreover, the reaction seems to be reversible. Both aldonic and saccharic acids exhibit this phenomenon as do the fully methylated γ - and δ -lactones.³²



A similar simple inversion has been observed on treating certain of the methylated sugars with dilute alkali.

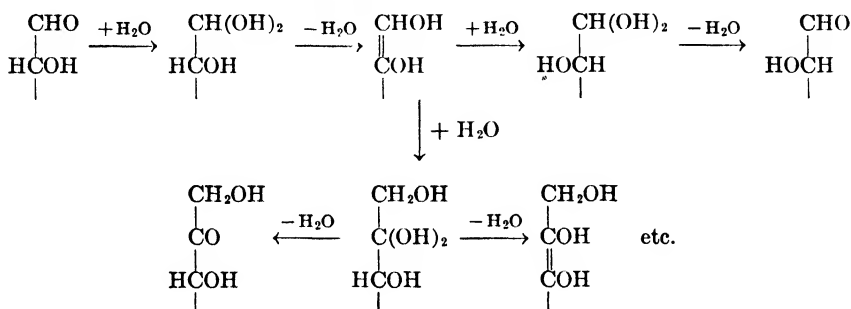
Not all reactions of this type are so simple, however, and if the free sugars be employed instead of the acids then the changes usually become much more complicated. In one of the simple cases, where glyceraldehyde is treated with anhydrous pyridine, a reversible conversion to dihydroxyacetone has been observed.



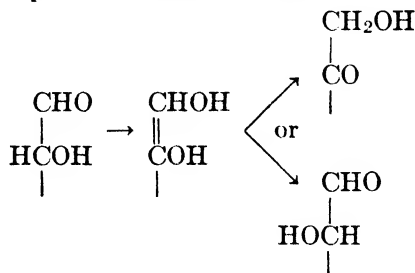
On treatment of glucose, however, with even as mild a reagent as saturated lime water, the first products are apparently mannose and fructose, but if the reaction be allowed to continue then a host of other products is formed. The nature of these substances and the mechanism of their formation have been extensively investigated, but they are still

³² Fischer, *Ber.*, **23**, 799 (1890); Haworth and Long, *J. Chem. Soc.*, 345 (1929); Hedenberg and Cretcher, *J. Am. Chem. Soc.*, **49**, 478 (1927).

not fully understood. Nef, following Wohl, Lobry de Bruyn, and Alberda van Ekenstein, argued for the enol (p. 1441) as being the intermediate in the alkaline reactions of the carbohydrates, and did extensive research in corroboration of this idea.³³ This theory assumed the enol to be formed by alternate addition and removal of the elements of water, the change being considered progressive.



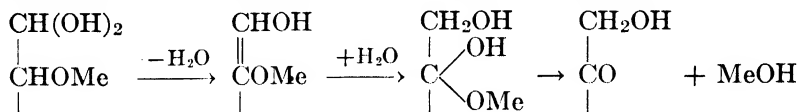
This mechanism accounts adequately for the known formation of glucose, fructose, and mannose from any one of the three used as starting material, as well as for the supposed presence of glucose (3-ketohexose) and the kindred products which have been claimed as being formed. It may be considered the classical or basic theory of these changes. However, when Lewis and his co-workers³⁴ attempted to apply this same mechanism in the methylated sugar series they met with difficulty. Working under conditions which led only to the simplest changes, with little or no saccharinic acid formation, Lewis and Wolfrom studied the effect of alkali on tetramethylglucopyranose. True equilibrium was apparently established between the glucose and mannose derivatives, but they observed no ketose formation and on this basis argued that the views of Nef and Lobry de Bruyn should be replaced by the simpler concept of enolization. Thus:



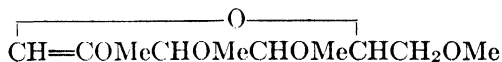
³³ Nef, *Ann.*, **335**, 191 (1904); **357**, 214 (1907); **376**, 1 (1910); **403**, 204 (1914).

³⁴ For most recent publication and earlier references see Loder with Lewis, *J. Am. Chem. Soc.*, **54**, 1040 (1932).

The changes predicted for the free sugars would be identical on either basis, but in the case of a methylated sugar Lewis considers that further enolization is blocked by the methyl group, owing to its non-mobility. It is his view that, on the basis of the Nef theory, water would be added, forming a hemiacetal which would readily lose methyl alcohol, and that subsequent changes would be similar in character.

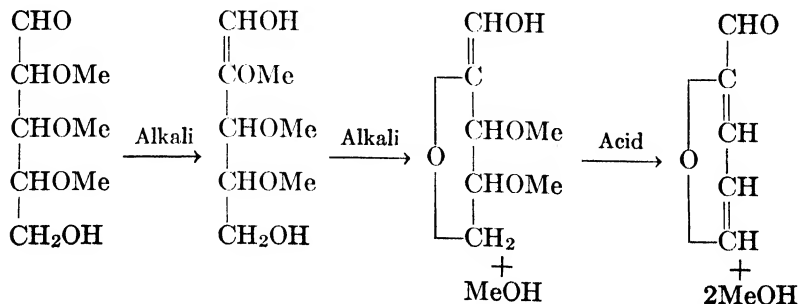


Against this it may be argued that this form of writing the reaction ignores the possibility of a lactol ring, and that if the ring form is considered then the postulated removal of the elements of water would give rise to tetramethyl-1,2-glucoseen:

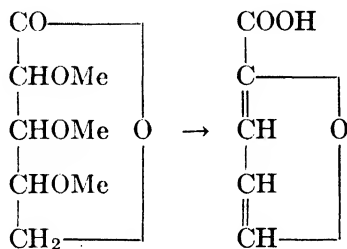


This is a type of substance whose reactions have not as yet been established.

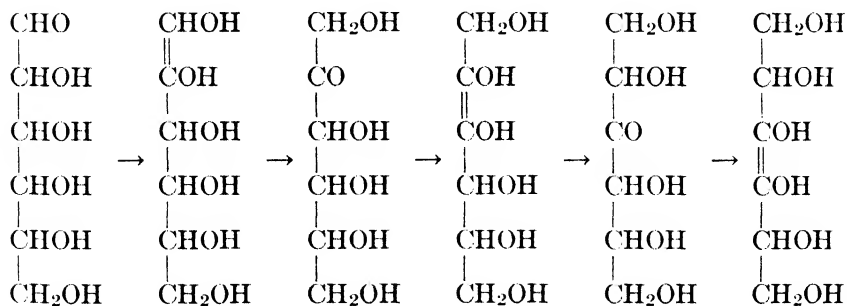
In these studies on the methylated sugars it was observed that the apparent aldose content, as determined by hypoiodite titration, increased to above 100 per cent but was reduced to normal on acidification. This was interpreted as proving that, in the methylated sugars, the assumed enediol has a tangible existence and that, by consuming more than one atom of oxygen per mole during oxidation, it is responsible for the high analytical figures. Similar results were secured with the methylated pentoses, but here further complications arose, as methyl alcohol was split off and furfural was formed. It was found that the proportion of furfural increased with increasing "high iodine" value and also that the amount of methyl alcohol split off on acidification was about double that liberated in alkaline solution. This led to the formulation of a mechanism for the reaction as follows:



In this connection there may be mentioned Hirst's observation that when 2,3,4-trimethyl- δ -xylonolactone was heated with aqueous pyridine in the usual fashion in order to produce epimerization, the major product of the reaction was not the epimeric lyxonolactone, but instead was furancarboxylic acid. In this reaction no acid treatment is required to cause elimination of all methyl groups.

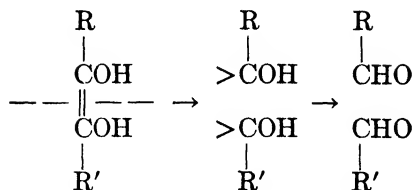


In all the investigations of Lewis, the conditions as mentioned above were so chosen as to lead exclusively to the simplest changes. Evans and his collaborators, on the other hand, have done a vast amount of work under more drastic conditions, leading to more deep-seated changes in the molecule.³⁵ In these studies, glucose, fructose, and mannose were found to react analogously, and the products were formed in roughly the same amounts in each instance. The experiments were therefore interpreted on the assumption that the first product is the common enol, which then either undergoes scission or is attended by migration of the double bond farther down the chain. Thus:

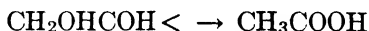


Scission of each of these enediols was also assumed, with subsequent rearrangement of the fragments.

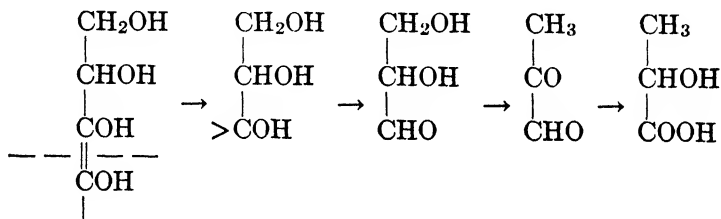
³⁵ For most recent publication and earlier references see Arnold and Evans, *J. Am. Chem. Soc.*, **58**, 1950 (1936).



The intermediates above are written in the Nef methylenic form to suggest their reactive nature, for although rearrangements such as



were assumed, experimentally no acetic acid was formed from glycolic aldehyde under similar conditions. Glyceraldehyde and dihydroxyacetone, on the other hand, did actually give rise to lactic acid as well as to pyruvic aldehyde (isolated as the osazone) although the yields were far from quantitative. These last-named substances may be included in the above scheme as follows:



as, quite obviously, may a great number of isomeric pentoses and tetroses.

In addition to the studies outlined above, Evans and his collaborators have extended the investigations to several other aldoses and disaccharides and to hexosediphosphate. They considered that all the quantitative results were in accord with the general scheme outlined above and substantiated it in all important respects.

Before leaving the subject it seems well to point out certain less-emphasized phases of the problem. First of these is the question of conversion of aldehydic intermediates into corresponding acids, for example, the assumed production of acetic acid from acetaldehyde, or of formic acid from methylenol ($=\text{CHOH}$). It is clear that both of these changes involve an oxidation, which in turn demands intervention of atmospheric oxygen or else simultaneous reduction of some other product. In either event it would lead to the production of a large series of compounds which have been disregarded in the original scheme. It

would seem necessary to be doubly cautious in considerations involving any products which do not have the empirical formula $(\text{CHOH})_n \pm x\text{H}_2\text{O}$. Actually Evans (private communication) has found that in a nitrogen atmosphere the peak of formic acid formation disappears, and he inclines to the view that even more vigorous exclusion of traces of oxygen is necessary.

A second assumption which appears to have been accepted in most studies of this sort is that of the reversible nature of the various reactions. In dealing with compounds of this type in which the free energy differences are small, it is frequently possible to produce, at will, either forward or reverse reactions depending upon concentrations and conditions. This, in turn, has been taken as indication of true reversible reaction (in the physicochemical sense), and this has been in fact frequently assumed, either explicitly or implicitly.³⁶ It is evident that thermodynamic equilibrium demands an identical final composition of the mixture, no matter which component is used as starting material, yet in the many experiments which have been performed this seems not to be the tendency. In general the initial component predominates while the other products appear to form in almost random ratio. The extenuating circumstance in most of these experiments is that saccharinic acids frequently form and thus decrease the alkalinity. Also, as Evans has pointed out, other acids may be formed and thus disturb the equilibria. The need for many additional data on the subject of true reversibility is evident.

The final and perhaps the most important assumption which needs scrutiny is that concerning scission of the enol forms.³⁷ That ethylenic linkages are reasonably susceptible of rupture by oxidants appears to be adequately established, but simple hydrolytic scission, such as that assumed in the mechanisms above, is on a much less secure experimental basis. It would appear desirable to have more extensive data on simple non-oxidative cleavage of enediols before accepting, without reservation, mechanisms based on this type of reaction.

Saccharinic Acid Formation. It has been shown above that the simplest effect of alkali on a sugar is the catalysis of mutarotation, the next is enol formation and epimerization, while more deep-seated changes are those of migration of the double bond and cleavage into smaller fragments. Accompanying these last reactions is still another, that of intramolecular oxidation and reduction (or rearrangement), leading to the formation of the so-called saccharinic acids. These are respectively:

³⁶ Nef, *Ann.*, **403**, 206 (1914); Kusin, *Ber.*, **69**, 1041 (1936).

³⁷ Evans *et al.*, *J. Org. Chem.*, **1**, 1 (1936); Schmidt, *Ber.*, **68**, 60 (1935); Neuberg, *Ber.*, **68**, 505 (1935).

- (a) Metasaccharinic acids $\text{CH}_2\text{OHCHOHCHOHCH}_2\text{CHOHCOOH}$ (eight possible hexonic)
- (b) Isosaccharinic acids $\text{CH}_2\text{OHCHOHCH}_2\text{COH} \begin{cases} \text{COOH} \\ \text{CH}_2\text{OH} \end{cases}$ (four possible hexonic)
- (c) Saccharinic acids $\text{CH}_2\text{OHCHOHCHOHCOH} \begin{cases} \text{COOH} \\ \text{CH}_3 \end{cases}$ (eight possible hexonic)
- (d) Parasaccharinic acids $\text{CH}_2\text{OHCHOHCOH} \begin{cases} \text{COOH} \\ \text{CH}_2\text{CH}_2\text{OH} \end{cases}$ (four possible hexonic)

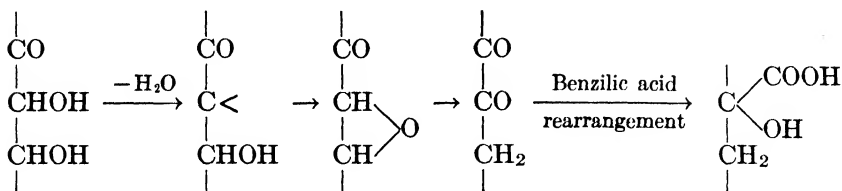
The formula in each case is $\text{C}_6\text{H}_{12}\text{O}_6$, so that each of these acids is isomeric with the parent hexose. Certain of the corresponding saccharinic acids from pentoses have been described by Nef, but these are analogous in character. Of the twenty-four possible isomers listed above, only a few have been described:

- (a) one or two metasaccharinic acids from galactose or lactose and two from glucose;
- (b) one or two isosaccharinic acids from maltose, lactose, or cellobiose but not from glucose or galactose;
- (c) one saccharinic acid from glucose or mannose;
- (d) one parasaccharinic acid from galactose or lactose.

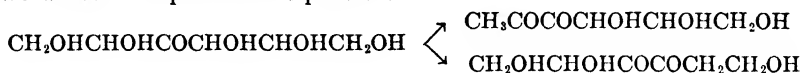
The mechanism of the formation of these substances has been the subject of extensive research but is still incompletely understood. The earliest attempt to account for their formation was that of Kiliani, who assumed that lactic acid and glyceraldehyde, formed from the sugar under the influence of alkali, were recondensed to give the saccharinic acids. By assuming condensation of other acids and aldehydes this mechanism was extended by Windaus to include all the isomeric saccharinic acids, but the theory has received little experimental support.

The most extensive investigations in the field were made by Nef, with his co-workers, in a series of classical researches. Based on the earlier work of Lobry de Bruyn, a progression of the carbonyl group down the carbon chain was postulated and the various ketoses thus formed were then assumed to undergo internal oxidation and reduction leading to the formation of desoxy diketo compounds. A benzilic acid rearrangement (p. 756) of these substances gave rise to

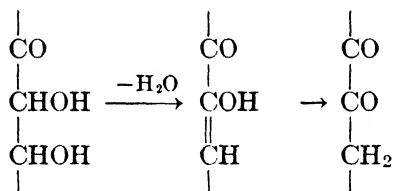
the various saccharinic acids. Thus, as written by Nef, who assumed the reactive methylenic intermediates:



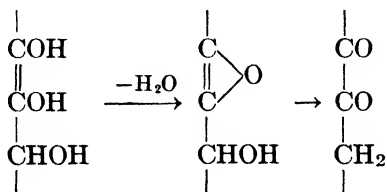
If this same scheme be followed, starting with the aldehydo-, 2-keto-, and 3-ketohexoses, it will be found that the first gives rise to the meta-saccharinic acids, the second to the isosaccharinic, and the third, where two diketo compounds are possible



to both saccharinic and parasaccharinic acids. In the above scheme the formation of the diketo compounds might equally well be based on a selective removal of the elements of water followed by ketonization, as proposed by Lewis:

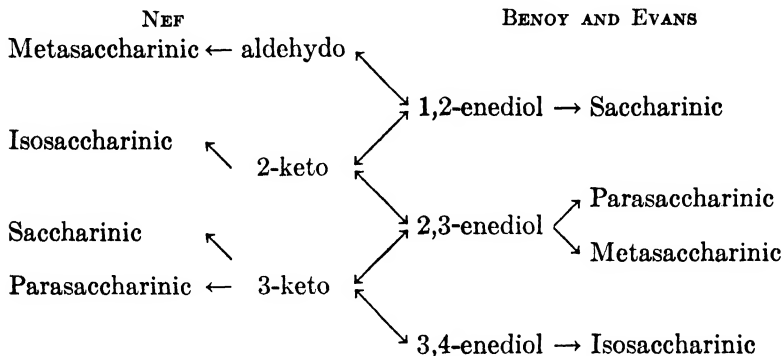


In recent years, Benoy and Evans have proposed a modification of Nef's scheme, based on their emphasis of the enediols as intermediates in these mechanisms.³⁸ Thus the same enediols which they postulated to account for the isomerizations produced by alkali serve as intermediates in the saccharinic acid formation, and the one mechanism accounts for both types of reactions. These authors assumed an isomerization of the enediol as follows:



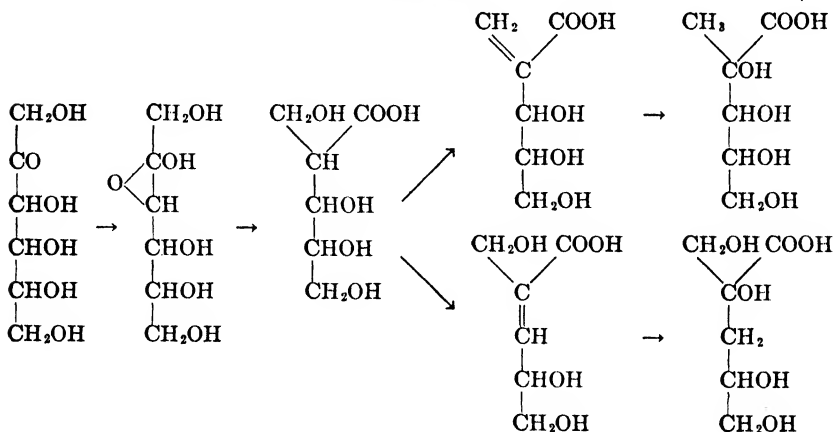
³⁸ Benoy and Evans, see *J. Am. Chem. Soc.*, **48**, 2675 (1926).

and the diketo derivative thus formed could undergo the benzilic acid rearrangement as assumed by Nef. The major difference between these two theories derives from the precursors which are assumed, as may be seen in the following scheme:



It would seem that quantitative studies on partially substituted hexoses or on the disaccharides might serve to decide between these two theories.

In recent years a further attempt to elucidate the mechanism of the formation of the saccharinic and isosaccharinic acids was made by Ohle. This author started from fructose, for example, and assuming a pinacol rearrangement, followed by selective removal and addition of water, formulated the desired substances.^{38a} Thus:

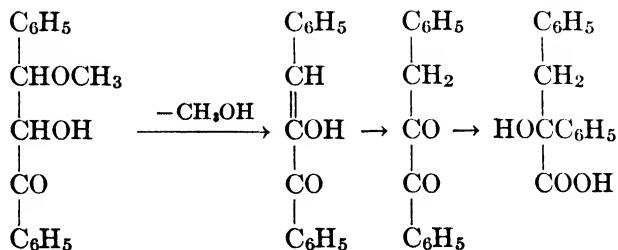


This mechanism can not be applied to the metasaccharinic and parasaccharinic acids.

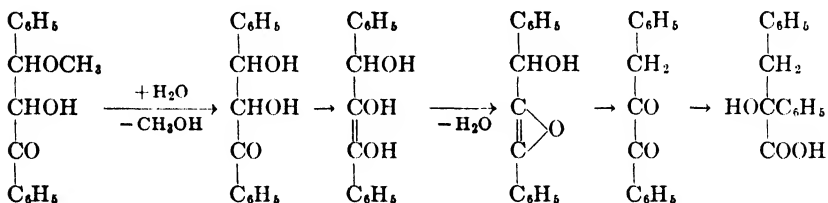
There may also be mentioned the observation of Nicolet that α -hydroxy- β -methoxy- β -phenylpropionophenone, on treatment with alkali,

^{38a} Ohle, *Ergeb. Physiol.*, **33**, 694 (1931).

gave α,β -diphenyllactic acid.³⁰ The author explained this on the basis of a benzilic acid transformation of the hypothetical diketone and suggested that the initial reaction (an "aldol dehydration") made a revision of Nef's theories necessary.

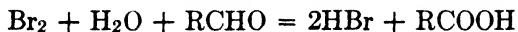


Evans (private communication), however, points out that, if the removal of methyl alcohol be assumed as the first step, the reaction may be represented as follows:



and thus included in the general mechanism outlined above.

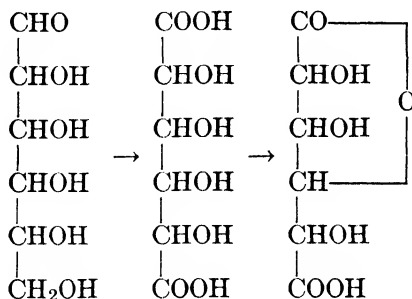
Oxidation. In this discussion of oxidation, as in that of isomerization, it is convenient first to consider the reactions in *acid* medium since they are of less involved nature. In the presence of strong acids the results are complicated by the isomerizations which lead to the production of furfural derivatives, levulinic acid, and the humic acids. With bromine, on the other hand, such isomerizations are reduced to a minimum, and the reaction with aldoses is largely confined to simple oxidation to the corresponding acid:



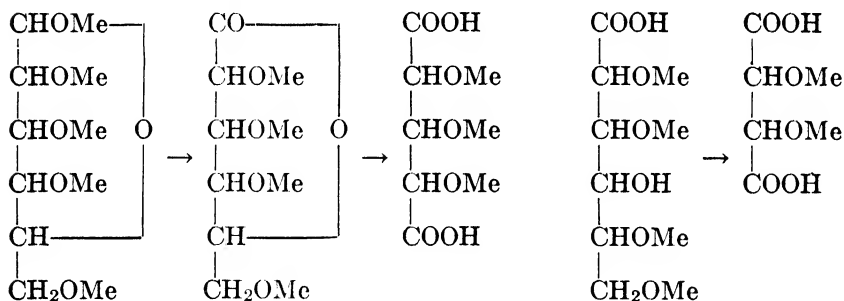
A similar conversion is achieved by using hot dilute nitric acid, and the major product is again the aldonic acid. This same reagent produces selective oxidation of the primary alcoholic group adjacent to the reducing carbon in the case of ketoses, and has been found a useful preparative method for the 2-keto aldonic acids which are intermediates in the ascorbic acid synthesis.

³⁰ Nicolet, *ibid.*, **53**, 4458 (1931). See Nef, *Ann.*, **376**, 3 (1910).

Boiling concentrated nitric acid oxidizes both the terminal carbons and gives the dicarboxylic acids (saccharic acids) most often in the form of their mono- or dilactones. Thus:



This same reagent is frequently used in structural determinations involving the methylated sugars, as they are attacked at the point of the lactol or lactone ring, the position of which is indicated by the nature of the oxidation products. For example,

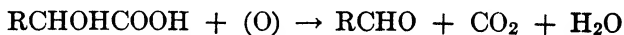


The method is similarly employed to determine the position of substituents, by fully methylating the substance, removing the substituent, oxidizing, and determining the nature of the oxidation products.

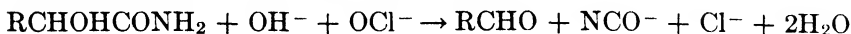
An oxidation which has preparative significance is that with hydrogen peroxide in the presence of iron catalysts. With ferrous salts both aldoses and 2-ketoses are converted to the osones, while fragments of the molecule appear as by-products in the form of the acids:



With ferric iron, notably colloidal ferric hydroxide, as catalyst the method becomes a useful one for the preparation of the aldoses of one less carbon from the aldonic acids:

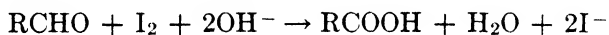


A similar oxidation has been achieved electrolytically, and a further special case of the same general reaction is the action of hypochlorites or hypobromites on the sugar acid amides:



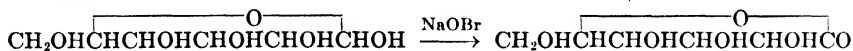
To Weerman is due the observation that this reaction may be used to prove substitution on the second carbon atom of aldoses, for formation of isocyanates does not then take place.⁴⁰

In *alkaline* oxidations the various isomerizations which have already been discussed must also be taken into consideration. With weakly alkaline reagents, and at low temperatures, isomerization may be negligible, but with the more alkaline oxidants, particularly when used hot, it may predominate. An example of the first class is oxidation with hypiodites, usually at room temperature or below. Simple oxidation of aldoses is observed, and under controlled conditions of alkalinity and temperature, ketohexoses are negligibly affected and the method is not only specific, but quantitative as well.



An oxidation, similar to this, has been achieved by Isbell who performed an electrolytic oxidation in the presence of small amounts of bromides. Here the bromide may be considered as being continuously oxidized to the hypobromite, and this in turn being continuously reduced by the aldose with the formation of the aldonic acid. A by-product of this reaction with glucose is 5-ketogluconic acid.^{40a} Oxidation of glucose directly with barium hypobromite leads to the production of a considerable amount of this 5-ketogluconic acid.^{40b}

Of theoretical importance in connection with the hypobromite oxidation is the conclusion of Isbell, who has presented evidence showing that there is a difference between the α - and β -forms of the aldoses as regards the rate of their oxidation by this reagent.⁴¹ The same author also believes that the sugars are directly oxidized by this reagent from their lactol to their lactone forms, the ring being unchanged in the process.⁴²



⁴⁰ Weerman, *Rec. trav. chim.*, **37**, 16 (1917). See Ault, Haworth, and Hirst, *J. Chem. Soc.*, 1722 (1934).

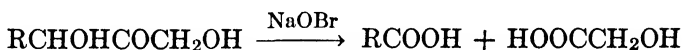
^{40a} Cook and Major, *J. Am. Chem. Soc.*, **57**, 773 (1935).

^{40b} Reichstein and Neracher, *Helv. Chim. Acta*, **18**, 892 (1935).

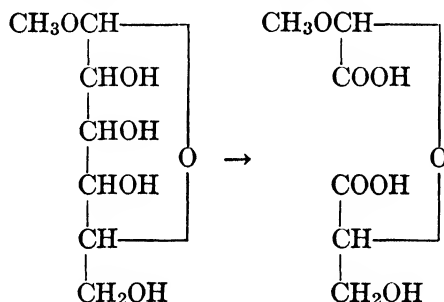
⁴¹ Isbell, *J. Am. Chem. Soc.*, **54**, 1692 (1932).

⁴² Isbell and Hudson, *J. Research Nat. Bur. Standards*, **8**, 327 (1932); Isbell, *ibid.*, p. 615.

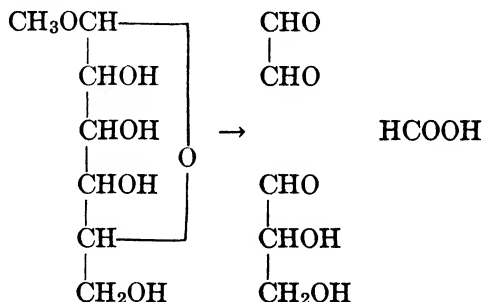
Another reaction of hypobromites is the more vigorous treatment of ketoses which cleaves the molecule at the carbonyl group. The same result is secured by using mercuric oxide in alkaline solution:



There has been recently described a most important reaction which involves the treatment of glycosides with hypobromites and leads to the complete elimination of the third carbon from the molecule, while the remainder is obtained as a mixed acetal:⁴³



This observation recalls earlier studies on the oxidations produced by periodic acid and lead tetraacetate.⁴⁴ These reagents are notable in that oxidation of the saturated sugars occurs only when two adjacent hydroxyls are free, so that oxidation of pyranosides would be expected between carbons two and three, or between carbons three and four. It was experimentally established that the actual oxidation is in both these places. The third carbon is eliminated as formic acid and two aldehyde groups are formed:

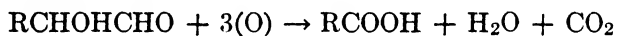


⁴³ Jackson and Hudson, *J. Am. Chem. Soc.*, **58**, 378 (1936).

⁴⁴ Karrer and Pfäehler, *Helv. Chim. Acta*, **17**, 766 (1934); Malaprade, *Bull. soc. chim.*, [4] **43**, 683 (1928); [5] **1**, 833 (1934); Hérissé, Fleury, and Joly, *J. pharm. chim.*, [8] **20**, 149 (1934).

Finally it was shown that preliminary oxidation by periodic acid, followed by oxidation with bromine water, gave the same result as the original oxidation with hypobromites but the yield was 65 per cent as compared with 15 per cent for the one step oxidation.

Many of the quantitative analytical reagents used for sugar determinations are more or less alkaline in reaction, and being used hot, produce fairly extensive isomerization. Sobotka has shown that in the series of methylated sugars, oxidation by a typical sugar reagent diminishes progressively as the methyl groups are moved toward the reducing carbon.⁴⁵ Thus 3-methylglucose is much less reducing than glucose itself, and 2,3-dimethylglucose has very little reducing action. This observation has been confirmed with several of the monomethylglucoses and has been explained on the basis of isomerizations leading to the formation of non-reducing products such as the saccharinic acids. However, it is to be noted that even in the case of free glucose, in the normal oxidation time (by which time the reaction is approaching a maximum) only three atoms of oxygen have been consumed. This is equivalent to the production of the acid of one less carbon:



so that it is surprising that 3-methylglucose should be so much less reducing. Although this is certainly in part due to the isomerizations mentioned above, it appears possible that it may also be due to the prevention of mid-chain oxidations (like that with hypobromite) by the presence of the stable substituted groups.

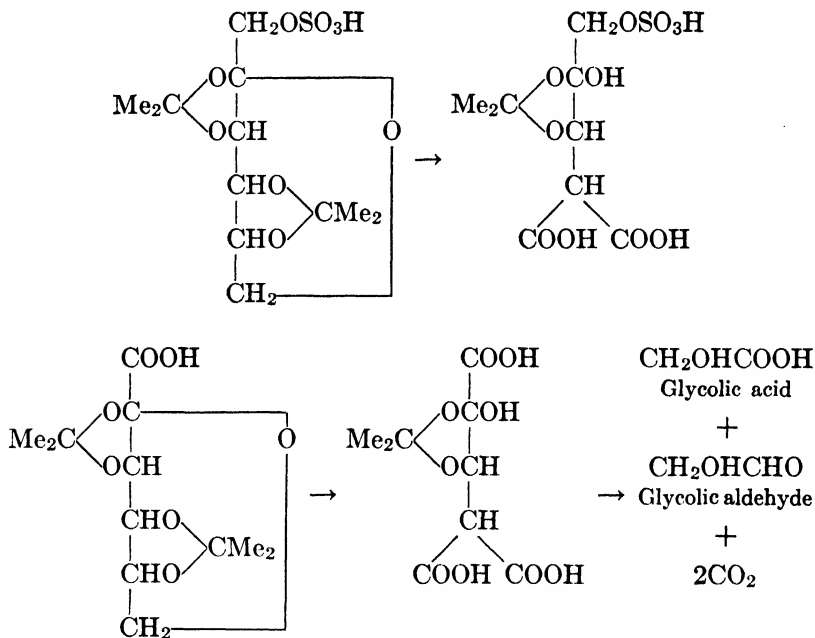
This oxidation by alkaline reagents has been extensively studied by Nef³³ and more recently by Evans,³⁵ and these authors interpret the results on the basis of the intermediates produced by the action of alkali, and their subsequent reaction with the oxidant. Evans' emphasis is upon the enediols as intermediates, and it was his conclusion that the quantitative results were in satisfactory agreement with the assumption of cleavage and oxidation of the various enediol forms.

In the discussion above there have been considered only a few of the many reagents and reactions which have been studied, and of which there are an enormous number. Catalytic oxidation, oxidation by air, by peroxides, and by salts and oxides of numerous metals have been extensively studied. They are omitted here for the reason that in general they are similarly explained and usually permit qualitative if not quantitative interpretation on the assumption of enediols and their reactions. Two reagents which may be mentioned are the peracids and

⁴⁵ Sobotka, *J. Biol. Chem.*, **69**, 267 (1926).

lead tetraacetate which are used for the oxidation of substances having double bonds in the molecule. As mentioned above, this last reagent has been found of theoretical and preparative use since it produces oxidation of the saturated sugars only if there are two adjacent unsubstituted hydroxyls. One final reagent which has found some application in structural determinations is silver oxide in aqueous solution.⁴⁶ There are indications that in the methylated sugars this reagent may produce oxidation only at the point of attachment of the lactol ring, although this has not as yet been firmly established.

As a final consideration in this section may be mentioned the work of Ohle and his collaborators,⁴⁷ directed towards securing "models" for the biological breakdown of carbohydrates. Working with fructose derivatives, they found as intermediates branched-chain dicarboxylic acids which they called furtonic acids. Thus β -diacetonefructose-1-sulfate, on oxidation with permanganate, gave β -monoacetone-*l*-furtondicarboxylic acid 1-sulfate, while β -diacetonefructose-1-carboxylic acid gave β -monoacetone-*l*-furtontricarboxylic acid. The last, on acid hydrolysis, yielded glycolic aldehyde, glycolic acid, and two molecules of carbon dioxide:



⁴⁶ See, for example, Freudenberg, *Ber.*, **59**, 836 (1926); Micheel, *Ber.*, **63**, 347 (1930); Levene and Compton, *J. Biol. Chem.*, **112**, 775 (1936).

⁴⁷ Ohle, Contsicos, and Gonzalez, *Ber.*, **64**, 1759, 2804, 2810 (1931).

Similar reactions were observed with α -diacetonelfructose-3-sulfate and with the corresponding phosphoric esters. With glucose, on the other hand, it was found that monoacetoneglucose-3-sulfate on oxidation gave the 3-sulfate of monoacetoneglucuronic acid and not a furtonic acid.

In spite of the very interesting nature of these reactions, considerable additional information will be needed before the results can be applied in any biological connections.

Fermentations

Alcoholic. One of the most important biological processes, and one of the most extensively studied, is the metabolism of carbohydrates. The best understood of these reactions is alcoholic fermentation, and, inasmuch as it may be used as a starting point for the discussion of almost all kindred processes, it may be considered at some length.

Live-yeast fermentation is confined to certain disaccharides, one of the two monosaccharides, a few hexoses, α -glucosan, 5-ketofructose, and the trioses, and on this basis it has been stated that only such sugars ferment as contain three or a multiple of three carbon atoms. In general it appears that the disaccharides undergo preliminary hydrolysis to the hexoses, although some evidence exists to show that this is not invariably true and that they may be fermented directly. The trioses, moreover, are fermented only slowly and may perhaps undergo a preliminary conversion, the exact nature of which will be discussed later. In any event the significant fermentation is that of the hexoses, and of these only *d*-glucose, *d*-mannose, *d*-fructose, and (by specially cultured yeasts) *d*-galactose are utilized. Introduction of any substituent whatsoever* has been found invariably to prevent fermentation.

In live-cell fermentation it was found that a quantitative "balance-sheet" could be prepared in which, with fair precision, the fermentation is described by the equation:



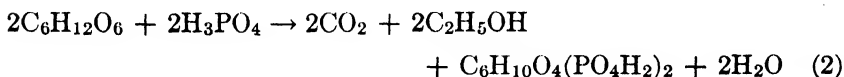
* Exceptions to this statement are the glycosides, but in general they are hydrolyzed prior to fermentation.

† From data given in Parks and Huffman, "The Free Energies of Some Organic Compounds," Chemical Catalog Co., New York (1932), it may be calculated that for this reaction (CO_2 at 1 atm., $\text{C}_2\text{H}_5\text{OH}$ and $\text{C}_6\text{H}_{12}\text{O}_6$ in 1 molal solution)

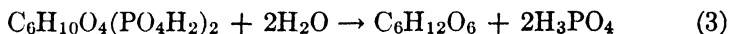
$$\frac{(\text{C}_2\text{H}_5\text{OH})^2[\text{CO}_2]^2}{(\text{C}_6\text{H}_{12}\text{O}_6)} = 10^{40} \quad (\text{approx.})$$

In view of this fact the irreversibility of fermentation in actual practice is not surprising.

However, working with the fermentation enzyme, Harden,⁴⁸ in his brilliant researches, made the astonishing discovery that inorganic phosphates are involved in the enzymic reaction which is more nearly represented by the equation:



Harden was able to isolate a large proportion of the organic phosphate required by this equation, and to show that the dynamics of the reaction were in essential accord with this mechanism. The fact that the simpler equation (1) applied in the live-cell fermentation was explained by assuming that in the latter a mechanism exists for the rapid hydrolysis of the hexosediphosphate, thus regenerating inorganic phosphate and fermentable hexose:



The net result of (2) and (3) is (1).

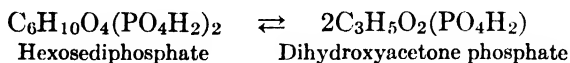
Such hydrolysis does, in fact, occur in the enzymic system but at so low a rate that the diphosphate accumulates during rapid fermentation. It is of interest in this connection that the addition of arsenates or arsenites increases the rate of enzymic fermentation, which, in special cases, may approach that of the living yeast equivalent to the amount of enzyme used. This has been explained by assuming that arsenates increase the rate of reaction (3), regenerating inorganic phosphate more rapidly and thus accelerating reaction (2).

Another significant fact, which must receive consideration, is derived from experiments on certain enzyme preparations which show a delayed starting or induction period, followed by normal fermentation. It has been found that certain of the phospho esters, but especially the hexosediphosphate, in very low concentration, reduce or entirely abolish this induction period so that fermentation starts almost immediately. Nothing in the preceding reactions permits prediction of this surprising result, which seems to indicate the diphosphate to be some sort of intermediate in the fermentation mechanism. Against this latter view is the fact that the diphosphate exhibits a very low rate of enzymic fermentation, whereas a true intermediate should ferment at least as fast as the parent hexose under equivalent conditions. To reconcile these two facts, recourse was usually had to the rather unsatisfactory explana-

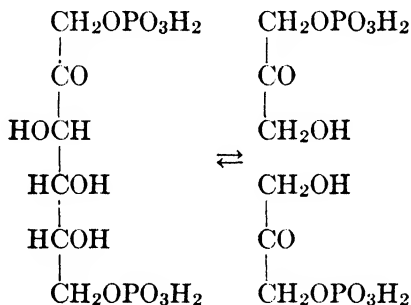
⁴⁸ See Monograph, "Alcoholic Fermentation," by Harden [Longmans, Green, and Co., London (1932)].

tion that the diphosphate was liberated in an "active" state which had a higher rate of fermentation than the "stable" forms which were isolated.

Within the past few years the problem has been entirely reopened and given a tremendous impetus by the important observations of Meyerhof and Lohmann, who found that an equilibrium is established with extraordinary speed between dihydroxyacetone phosphate and hexosediphosphate in the presence of yeast enzyme.⁴⁹



This constitutes one of the most significant observations thus far made in the field of carbohydrate metabolism, as here for the first time is a mechanism for securing the smaller triose units which have been so often postulated. Not only is considerable rearrangement involved in the reaction, but also an interchange of optically active and optically inactive material:



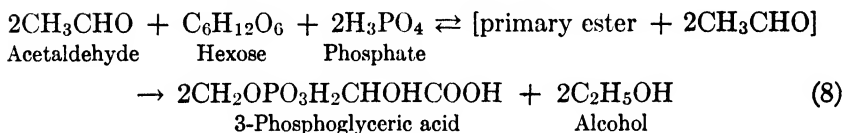
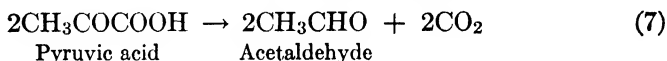
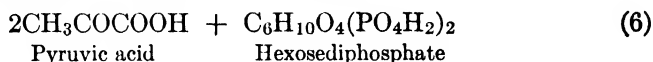
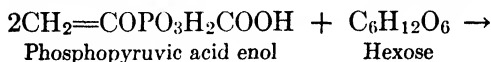
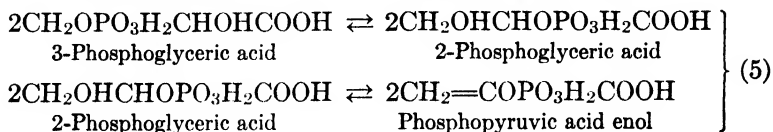
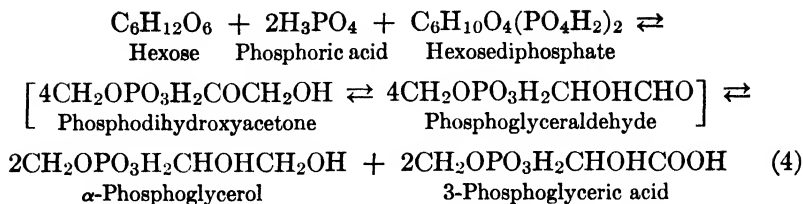
The change is reversible, and it is of great interest that true thermodynamic equilibrium is attained.

Following this major discovery, a new activity in this research field has brought to light a series of most remarkable reactions. Although the problem is in a transitional state, and although some of the present views may require revision later, it seems desirable to consider the current concept. This is perhaps best achieved by presenting the mechanism proposed by Meyerhof and Kiessling, which is as follows:⁵⁰

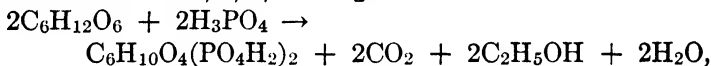
⁴⁹ Meyerhof and Lohmann, *Naturwissenschaften*, **22**, 134 (1934); *Biochem. Z.*, **271**, 89 (1934); **273**, 413 (1934); **275**, 430 (1935).

⁵⁰ Meyerhof and Kiessling, *ibid.*, **281**, 249 (1935); **283**, 83 (1935).

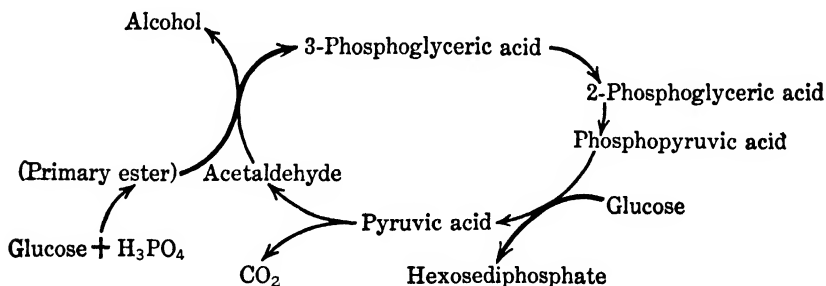
See also the earlier basic results of Embden. For example, Embden, Deuticke, and Kraft, *Klin. Wochschr.*, **12**, 213 (1933); Embden and co-workers, *Z. physiol. Chem.*, **230**, 1 (1934).



The summation of 5, 6, 7, and 8 gives



identical with reaction (2) discovered by Harden. In order to make clearer the sequence of events leading to this result, the above mechanism is given in diagrammatic form below:



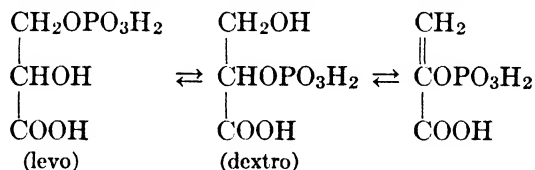
In this diagram there may be seen the utilization of glucose and inorganic phosphate and the production of hexosediphosphate, alcohol, and carbon dioxide, while the other products are formed and consumed in a cyclic manner.

In the mechanism as outlined in the equations above, the only hypothetical substances are the intermediate triose phosphates in reaction (4) which have not been actually demonstrated in this reaction, and the primary ester which is assumed in reaction (8). With these exceptions all the substances indicated have been isolated, and in many instances they have been made available synthetically. The products have usually been demonstrated by employing incomplete systems (such as one lacking coenzyme) or partially impaired enzymes (such as those poisoned by iodoacetate or fluoride) under which circumstances the intermediates accumulate and can be isolated.

The experimental basis for this mechanism is as follows:

Equation (4): Although hexosediphosphate alone is only slowly fermented by yeast juice, in the presence of glucose and inorganic phosphate it is very rapidly converted into equimolal quantities of α -phosphoglycerol and 3-phosphoglyceric acid. This presumably occurs through the intermediates, phosphodihydroxyacetone and phosphoglyceraldehyde, and there is evidence indicating the reversible interconversion of these two substances.

Equations (5): 3-Phosphoglyceric acid (which, incidentally, has also been prepared synthetically) undergoes a two-stage reaction, each step being reversible, whereby (levo)3-phosphoglyceric acid is converted into (dextro)2-phosphoglyceric acid. This in turn loses the elements of water and gives the phosphate of the enol form of pyruvic acid. This final product has also been prepared synthetically.



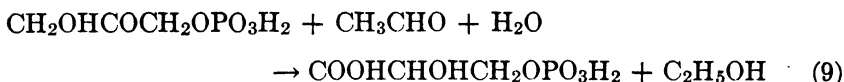
Equation (6): If glucose is added to either the natural or synthetic phosphopyruvic acid in the presence of the enzyme system, pyruvic acid and hexosediphosphate are formed by interchange of the phospho groups. This reaction is believed to be irreversible.

Equation (7): The pyruvic acid formed in reaction (6) is decarboxylated by the long-known enzyme *carboxylase*, and yields acetaldehyde and carbon dioxide. This reaction is irreversible in character.

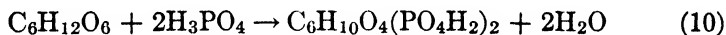
Equation (8): Acetaldehyde, glucose, and inorganic phosphate have been shown to react rapidly in the presence of hexosediphosphate and the yeast enzyme (through an assumed intermediate ester) reducing the aldehyde to alcohol and regenerating the original 3-phosphoglyceric acid. In this way the concentration of the latter is kept constant.

As will be seen from this discussion, the proposed mechanism rests on a fairly secure experimental basis. Moreover it includes certain other phenomena connected with fermentation which can not be discussed here. However, it can not be stated with certainty that the above is the actual sequence of reactions in normal, unimpaired fermentation and it is to be noted that certain of the proposed reactions [(8), for example] are of extremely high order. It seems probable that in such cases intermediate reactions occur which have not, as yet, been demonstrated. Moreover, although hexosediphosphate is written in all the above equations, Meyerhof intends this to include monophosphates as well, and this relationship is still obscure. Finally it is to be noted that the "primary ester," which is postulated above, is considered to be an unstable phospho ester, not identical with any of those already known. The search for an intermediate of this nature dates back to Harden's original discovery of the participation of phosphates in the fermentation system.

In addition to the studies discussed above, there must be mentioned the work of Schäffner and co-workers,⁵¹ who prepared mixtures of purified enzymes obtained from various sources, and who by use of these "synthetic" fermentation systems were able to produce the following reactions:

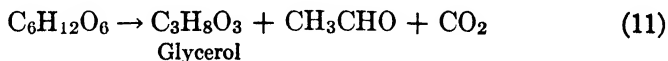


and



Reaction (9), by some obscure mechanism, induces reaction (10), and both are simultaneously inhibited by iodoacetic acid. These reactions, though not of immediate relationship to the mechanism above, nevertheless appear to be of considerable significance.

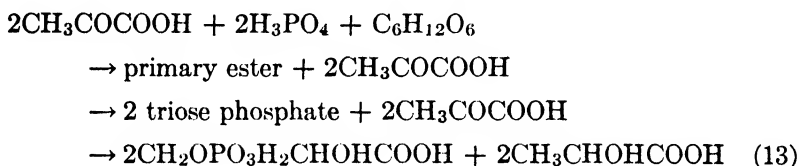
In addition to the simple fermentation (1), the mechanism of Meyerhof and Kiessling may also be employed to explain some of the older observations of Neuberg.⁴⁸ In an attempt to "fix," or remove from the reaction, possible aldehydic intermediates this author added sulfites or alkali to both live-yeast and enzymic fermentations and in this way was able to produce two reactions:



⁵¹ Schäffner and Bauer, *Naturwissenschaften*, **22**, 464 (1934); Schäffner, Bauer, and Berl, *Z. physiol. Chem.*, **232**, 213 (1935); **234**, 146 (1935).

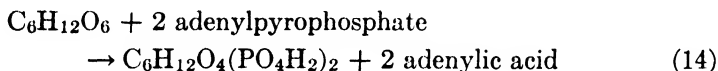
It will be noted that (12) would result by combining a "Cannizzaro" reaction, $2\text{CH}_3\text{CHO} + \text{H}_2\text{O} = \text{CH}_3\text{COOH} + \text{C}_2\text{H}_5\text{OH}$, with (11). The existence of an enzyme has in fact been demonstrated which accomplishes this type of change. Reaction (11) may be explained, on the basis of the Meyerhof-Kiessling mechanism, as resulting from removal of the acetaldehyde so that reaction (8) can no longer occur, and the 3-phosphoglyceric acid is therefore not regenerated. Thus, to replace it, (4) must proceed, and α -phosphoglycerol continues to be formed and to be converted to the glycerol indicated above.

Muscle Metabolism. Many of the data discussed above have been secured in studies on the closely related problem of muscle metabolism. This highly important mechanism has been included by Meyerhof and Kiessling in a similar scheme, in which reactions (4), (5), and (6) are the same and (7) and (8) are replaced by:



The phosphoglyceric acid for (5) is thus similarly regenerated, but the product of the reaction is lactic acid instead of alcohol and carbon dioxide.

An additional reaction which has been demonstrated in the muscle system is



This is of considerable interest in connection with the problems of coenzyme and hexosediphosphate formation, but its implications cannot be considered here.

Other Fermentations. The mechanism just described can quite obviously be employed to describe the important lactic acid fermentation, for the net initial reaction is the same:

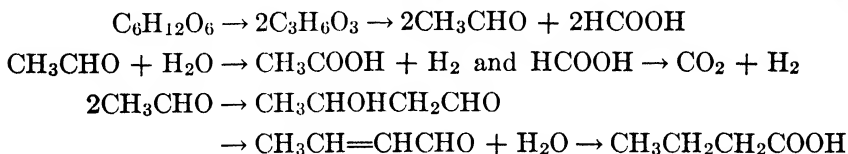


although in the muscle the lactic acid then undergoes further transformation. That the above mechanism is indeed applicable to the lactic acid fermentation is partially substantiated by experimental evidence, but this is not yet nearly so complete as that for alcoholic fermentation.

In view of the tremendous amount of research which has been required to advance the understanding of these well-known processes to its present stage, it is not surprising that in the less-explored fields

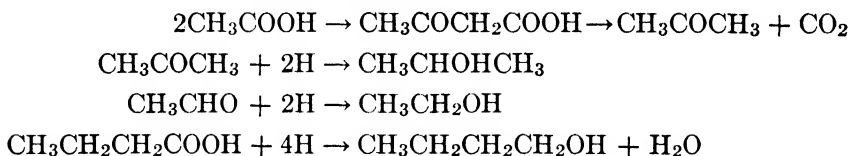
there should be little real knowledge. The ingenious and plausible mechanisms which might be written are largely without experimental verification and for some the enzyme systems have not even been isolated. Thus for the moment it seems desirable to postpone consideration of possible mechanisms until adequate data have been accumulated. Some of the commoner fermentations are indicated below, but in most of these reactions two or more processes appear actually to be taking place simultaneously, for the proportions of the various products may be changed by modifying the conditions. These are, therefore, to be considered as idealized equations which serve chiefly to indicate the nature of the products formed.

Butyric Acid Fermentation. A suggested mechanism is the following:



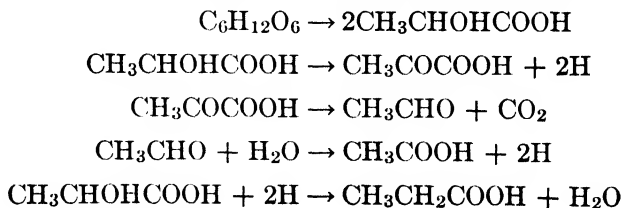
This mechanism accounts for the main products of the reaction which are butyric and acetic acids, carbon dioxide, and hydrogen, while a trace of formic acid is always present. The intermediate triose postulated above is presumably formed by the same sort of mechanism as in alcoholic fermentation and may actually be a phospho ester.

Butyl Alcohol and Acetone. The mechanism is like that above, with the addition of the following reactions:



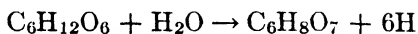
In this way, butyl alcohol, acetone, and ethyl and isopropyl alcohols, are added to the products above. The first two are the dominant reaction products, and the fermentation has considerable commercial utility for production of these substances.

Propionic Acid. The intermediate here appears to be lactic acid:



Propionic and acetic acids dominate, although other by-products are formed.

Citric Acid. The formation of a tribasic acid manifestly involves a very complex reaction, but the equation may be written in a simple form:



Acceptors for the hydrogen may be postulated in numerous ways.

Xylose Fermentation. Among the many pentose fermentations may be mentioned one in which xylose is fermented to the extent of 85-90 per cent to an equimolar mixture of acetic and lactic acids:



Oxidation by Acetobacter suboxydans. This organism produces dehydrogenations of the general type, $\begin{array}{c} | \\ \text{CHOH} \rightarrow \text{CO} + 2\text{H} \\ | \end{array}$. In this manner alcohol is converted to acetaldehyde, isopropyl alcohol to acetone, glycerol to dihydroxyacetone, sugar alcohols to ketoses, and gluconic acid to 5-ketogluconic acid. In the sugars the CHOH group adjacent to the primary alcohol group is converted to the ketose.

Oxidation by Acetobacter xylinum. The initial oxidations are identical with those above, but further oxidation of the products also occurs.

It is to be noted that in all these reactions the essential feature is the transfer of hydrogen by one or more mechanisms, and this may be considered as fundamental to all these biological processes. However, as in the alcoholic fermentation, the mechanism of this transport may prove to be highly complex.

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CHAPTER 18

CARBOHYDRATES III—CELLULOSE

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INTRODUCTION

Cellulose, the chief constituent of all living plants, is built up of glucose 'anhydride units ($C_6H_{10}O_5$), and this suggests that it owes its formation in the plant to a condensation of glucose units with loss of water. These units, it is now believed, are arranged in long chains, but how many units are linked together in the cellulose as it appears in the fiber cannot yet be stated with certainty. However, without doubt there are many, endowing this substance with all the characteristics of high-molecular-weight compounds. This fact, together with the fact that cellulose, in contrast to many other carbohydrates, appears in the form of an organized structure, complicates the course of the reactions which cellulose undergoes upon various treatments. Both these facts call for close consideration in order to understand the manifold and often strange phenomena which must be dealt with in the laboratory or in the factories in which cellulose is either the chief product or the raw material. On the other hand, these peculiarities—namely, its colloidal character and its fibrous structure, explain the enormous utility of cellulose for a great variety of purposes.

Cellulose may be obtained from any plant. The process of isolation is always the same in principle, that is, the non-cellulosic substances, for example lignin of wood, are removed by more or less drastic methods of treatment and subsequently the cellulose is obtained in its various structural forms, as hair (from cotton), as bast (from flax, etc.), and as fibers (from wood). The term fiber is now being used for all forms, however. Cotton is the most productive cellulose source for the textile industry; wood is the chief cellulosic raw material for the pulp and paper industry. Artificial fibers (rayon, cuprammonium silk, acetate silk, and other types) have for their sources both cotton and wood, though wood is assuming greater prominence.

The purest cellulose may be obtained from raw cotton. After fat, wax, and other soluble impurities have been removed by extraction with organic solvents, the cellulose is freed of other non-cellulosic substances, such as pectin, by careful treatment with dilute alkali, and, after washing, it is bleached slightly with hypochlorite or other bleaching agents. The resulting product is termed standard cellulose and may be used as such for experimental studies.*

Wood pulp may be obtained by cooking wood, in the form of small chips, with a solution of calcium or sodium bisulfite and free sulfurous acid (sulfite process), or with a solution of caustic soda (soda process),

* This is the American Chemical Society method. See Corey and Gray, *Ind. Eng. Chem.*, **16**, 853, 1130 (1924). See, also, Schwalbe, *Papier-Fabr.*, **24**, 769 (1926).

or with a solution containing, in addition to sodium hydroxide, a certain percentage of sodium sulfide originating from sodium sulfate (sulfate process).

The pulp so obtained retains a few per cent of lignin, varying amounts of non-cellulosic carbohydrates such as xylan, araban, and mannan, as well as a few per cent of resin and ash. By bleaching, the lignin and other coloring matter may be removed entirely, but the other non-cellulosic substances and the resin are more difficult to remove. Alkaline refining treatment reduces these substances to low percentages.

Pulp manufactured in this way may be used for scientific investigation after the remainder of the resin has been extracted with organic solvents and the ash content reduced by treatment with dilute mineral acids at a slightly elevated temperature.

The reactions which cellulose undergoes resemble those which are observed in the simple sugars. Since, however, of the aldehyde—that is, reducing—groups possibly all but one are involved in the linkings between the individual glucose anhydrides, cellulose lacks the pronounced reducing power of most of the sugars, and its chief reactions are based upon its hydroxyl groups. These react as in alcohols forming addition compounds with alkalis and certain complex salts; they react also to form alcoholates, esters, and ethers, and on oxidation are converted step-wise into aldehydic and into carboxylic groups. In the formation of esters and ethers the large cellulose molecule may be maintained more or less intact, but oxidation is often preceded or accompanied by hydrolysis at the bridges between the individual glucose anhydrides. Thus the outcome of oxidation is often merely a mixture of still intact cellulose with the low-molecular-weight products of hydrolysis and oxidation. The latter is termed either “hydrocellulose” or “oxycellulose,” depending upon which type of these products predominates in the mixture.

Because it is chiefly the hydroxyl groups which characterize the reactions of cellulose, it may be classified as an aliphatic alcohol and it may be expected to behave as such.

Primary means of clarifying the chemical constitution of cellulose are offered by the process of degradation, and of the various possible methods hydrolysis, preferably after methylation, is most suitable for determining the mode of linking between the individual glucose anhydride units. Gradual hydrolysis or acetolysis (that is, hydrolysis after acetylation) also leads to the isolation of a number of oligosaccharides, with six, five, four, and three glucose anhydrides linked together, before the disaccharide, cellobiose (p. 1464), and the end product, glucose (p. 1401) are reached.

X-ray analysis has become the primary means of investigating the

fine structure of cellulose and has definitely revealed its crystalline nature. It is assumed that within the geometrical unit cell the individual molecular chains are arranged parallel to each other and that this arrangement is laterally stabilized by secondary valence forces exerted by quadrivalent oxygen atoms. It is also assumed that the chain bundles are of varying length but of fixed breadth and thickness, and are arranged in hypothetical units called crystallites or micellae. These micellae, assumed to be held together by "tertiary" or "micellar" forces, build up the fibrillae, the first constituents of the fiber which may be detected under the microscope.

The complicated physical structure of cellulose causes the reactions to assume a heterogeneous character, in contrast to the homogeneous reactions exhibited by most other organic compounds.

THE FORMATION OF ALKALI CELLULOSE AND CELLULOSE ALCOHOLATES

Caustic soda solution containing about 18 per cent sodium hydroxide exerts a considerable swelling effect on cellulose, an effect which is used commercially in the process of mercerization. This phenomenon is distinctly exothermic, and it seems established that under the conditions of mercerization a chemical combination of the two components takes place which leads to a definite chemical compound of the formula $(C_6H_{10}O_5)_2 \cdot NaOH$.^{*} The compound is termed alkali cellulose or, more exactly, sodium hydroxide cellulose. There are possibly one or two molecules of water in chemical combination with the alkali cellulose.

The combination of two glucose anhydride units with only one molecule of sodium hydroxide has its parallel in the behavior of a number of simple alcohols, polyalcohols, and polyhydroxy compounds with metal hydroxides. Thus, for example, glycerol, erythritol, mannitol, and dulcitol undergo reactions to form addition compounds with metal hydroxides which may be regarded as complex compounds in the Werner sense.

It also appears quite possible that the reaction between cellulose and sodium hydroxide is one of alcoholate formation. This has been indirectly deduced from the so-called viscose reaction—i.e., the conversion of alkali cellulose by means of carbon disulfide into cellulose xanthate which, dissolved in dilute caustic soda solution, is used for the manufacture of artificial silk (viscose silk, rayon), films (cellophane), and so forth. This reaction appears to be analogous to the formation of xan-

^{*} This formula expresses the fact that the compound contains one molecule of sodium hydroxide per two glucose anhydride units of the cellulose chain. In this and like formulas throughout the chapter, the polymeric character of the cellulose portion is implied.

thogenic acid by the action of carbon disulfide on sodium alcoholate (p. 1548). Metallic sodium dissolved in liquid ammonia reacts with cellulose, one atom of sodium entering the molecule rather easily whereas two more react only slowly; since, however, not more than three sodium atoms enter the ($C_6H_{10}O_5$) unit, cellulose may be regarded as a trihydric alcohol.¹

It is interesting to note that the mercerizing effect usually secured at about 20° with a 17–18 per cent caustic soda solution is obtained at lower temperatures with less concentrated solutions, for example, at –10° a concentration of 6.5 per cent suffices. It must be assumed that the low temperature combination leads to the same alkali compound. It should be mentioned here that at 20° the maximum swelling effect is obtained with a 10–12 per cent sodium hydroxide solution.

The concept of a number of investigators that the reaction between cellulose and sodium hydroxide is one of simple adsorption according to purely physical laws² cannot be well understood in the light of x-ray (p. 1586) analysis, for in following the reaction, using sodium hydroxide of increasing concentration, x-ray analysis shows that true adsorption is dealt with only below a concentration of 9 per cent. Below this concentration the x-ray pattern of the fiber remains unchanged, but with increasing concentration the diagram begins to change and shows a very distinct alteration at a concentration in the neighborhood of 18 per cent.

More recent x-ray analysis seems to indicate that not one but a variety of alkali celluloses are formed in the course of treating cellulose with caustic soda solutions of increasing concentration. However, nothing definite can yet be said about their compositions or about the types of reactions,³ and the same seems to hold true as regards the various addition compounds which cellulose forms with ammonia.⁴

Alkali celluloses may also be obtained with the hydroxides of potassium, lithium, cesium, and rubidium. The first two combine as does sodium hydroxide, namely, with two molecules of cellulose, but cesium and rubidium combine with three. It is interesting to note that each hydroxide shows its maximum swelling effect upon cellulose at a definite concentration. This effect is greatest with lithium hydroxide and smallest with cesium hydroxide, and the maxima coincide with that

¹ Scherer and Hussey, *J. Am. Chem. Soc.*, **53**, 2344 (1931).

² Bancroft and Calkin, *J. Phys. Chem.*, **39**, 1 (1935); Calkin, "Colloid Symposium Monograph" (1936).

³ Schramek and Küttner, *Kolloid-Beihfte*, **42**, 331 (1935); previous literature also cited here.

⁴ Barry, Peterson and King, *J. Am. Chem. Soc.*, **58**, 333 (1936); see, also, Clark and Parker, paper presented before the Cellulose Division of the American Chemical Society at Chapel Hill, N. C., April, 1937. Hess and Gundermann, *Ber.*, **70**, 527 (1937); Meyer and Badenhuisen, *Nature*, **140**, 281 (1937).

concentration of the hydroxide solution at which formation of the alkali cellulose compound occurs.^{4a}

A number of strong organic bases such as trimethylsulfonium hydroxide and guanidinium hydroxide, as well as hydrazine, ethylenediamine, and tetramethylenediamine, also combine with cellulose in varying proportions.^{4b} Some of them dissolve cellulose.^{4c}

Alkali cellulose is very unstable, being easily decomposed by water into its components. The cellulose is regenerated with a number of physical properties changed. The term "cellulose hydrate" for the regenerated (mercerized) cellulose is derived from the earlier conception that the cellulose is regenerated from the alkali cellulose with one molecule of water chemically combined. This, however, is not so. The physical changes, due principally to the swelling under the influence of the alkalies, are indicated particularly by increased hygroscopicity, greater absorption capacity for dyestuffs, and greater reactivity in general, provided the cellulose hydrate is not subjected to much drying at too elevated temperatures. This increased reactivity of cellulose hydrate has also been demonstrated by the intensified action of enzymes, such as cellulase which leads to glucose.^{4d}

The physical changes are also reflected in the x-ray diagram of the cellulose hydrates; the lattice appears slightly deformed and widened, which might explain the greater reactivity of cellulose hydrate.^{4e}

The same physical changes are found in all cellulose preparations which are obtained by regeneration, for example, from solutions of cellulose in cuprammonium hydroxide and complex salts, and from esters.

It may be mentioned that the mercerizing effect (i.e., the phenomenon in its physical aspect) is also obtained by allowing strong inorganic acids, such as concentrated sulfuric or nitric acid, to act upon cellulose for a short time, the action being due to the swelling effect these acids exert.

As mentioned above, the formation of alkali cellulose plays an essential part in the viscose reaction. In order to obtain a solution which may be "spun" (forced through the very fine openings of a nozzle) without difficulties—that is, a solution of sufficiently low final viscosity—it is necessary to subject the alkali cellulose to a certain degree of degradation. This is termed "aging" and is brought about by allow-

^{4a} Heuser and Bartunek, *Cellulosechem.*, **6**, 19 (1925).

^{4b} Dehnert and König, *ibid.*, **5**, 107 (1924).

^{4c} Lieser, *Ann.*, **528**, 276 (1937); Bock, paper presented before the Cellulose Division of the American Chemical Society at Chapel Hill, N. C., April, 1937.

^{4d} Karrer and Illing, *Helv. Chim. Acta*, **8**, 245 (1925); Karrer, Schubert, and Wehrli, *ibid.*, **8**, 797 (1925).

^{4e} Mark, "Physik und Chemie der Cellulose," Springer, Berlin (1932), p. 215.

ing the alkali cellulose, after being freed of excess caustic soda by means of pressing or centrifuging, to stand at room temperature for approximately 72 hours. The aging process is characterized by: (1) a decrease in viscosity of the regenerated cellulose, e.g., in cuprammonium solution; (2) an increase in the solubility of the regenerate in an 8 per cent caustic soda solution; and (3) a slight increase in reducing power and a slight liberation of carbon dioxide. This carbon dioxide evolution, and the fact that these changes are much less pronounced when aging takes place with the exclusion of air and are marked when oxygen is given access, indicate that, chemically, aging is a process of oxidation. Besides, physical degradation of the micellar structure takes place. Both would explain the decrease in viscosity and the increase in solubility. The products of oxidation consist of aldehydes and acids, probably also aldehydo acids such as glucuronic acid^{4f} (p. 1447).

Hot alkalies do not exert the mercerizing effect; they dissolve the cellulose partly or completely, depending upon the concentration, temperature, and pressure. On complete solution of cellulose at high temperature and under high pressure, a variety of decomposition products of various sugars results, among which is found principally lactic acid.^{4g} W. L. Evans and his school have thrown much light on the mechanism of alkaline degradation of simple sugars and various di- and oligosaccharides.⁵

Fusion of cellulose with solid sodium hydroxide results in a far-reaching degradation of cellulose. The chief product is oxalic acid.

CUPRAMMONIUM CELLULOSE

Cellulose first swells considerably, then dissolves in a solution of cupric oxide in ammonia. This solvent, called "Schweitzer's reagent" or "cuprammonium" solution, is one of the very few solvents for cellulose from which it may be regenerated practically unchanged chemically.

It will be remembered that the complex base, copper-tetraammino hydroxide $[\text{Cu}(\text{NH}_3)_4(\text{OH})_2]$, contained in the cuprammonium solution, reacts with some polyalcohols like glycerol to form complex compounds. Cellulose, too, is capable of forming a complex addition compound with the base in which possibly two glucose units react with one copper atom

^{4f} Heuser and Schuster, *Cellulosechem.*, **7**, 17 (1926); Waentig, *Kolloid-Z.*, **41**, 154 (1927); Weltzien and zum Tobel, *Ber.*, **60**, 2024 (1927); Lottermoser and Schwarz, *Kolloid-Beihfte*, **42**, 408 (1935); Davidson, *J. Textile Inst.*, **23**, T. 95 (1932).

^{4g} Heuser, *Paper Trade J.*, **89**, T. 271 (1929).

⁵ Evans and collaborators, *J. Am. Chem. Soc.*, **53**, 4384 (1931); **54**, 698 (1932); also, "The Alkaline Degradation of Certain Oligosaccharides"; paper read before the Division of Organic Chemistry of the American Chemical Society in Pittsburgh, September, 1936. See also, Spengler and Pflannenstiell, *Angew. Chem.*, **48**, 475 (1935).

to form a copper alcoholate, two of which then unite with one molecule of copper-tetraammino hydroxide.

Cellulose in cuprammonium solution shows very pronounced levorotation, a phenomenon which is used for determining the degree of degradation of cellulose preparations.⁶ It also led Hess to develop the hypothesis that cellulose in cuprammonium solution is dissolved to monomolecular glucose anhydrides which are both chemically and kinetically independent and that cellulose itself represents an association of the monomolecular glucose anhydrides held together by forces other than primary valence forces.^{6a} According to more recent views it does not seem to be necessary to explain the rotatory power of the cuprammonium cellulose on the ground that the primary valence structure of the cellulose molecule has changed.

Cellulose may be regenerated from its solution by means of dilute acids, ammonium chloride, and many other salts, as well as by alkalies. No change can be recognized provided that regeneration is brought about not too long after dissolution and that the latter took place with careful exclusion of oxygen (air) and light.^{6b} However, in the presence of air, cellulose is very sensitive and undergoes partial oxidation. This change, which under these conditions becomes measurable after only a few minutes, is indicated by a decrease in viscosity and an increase in the solubility of the regenerate in 8 per cent caustic soda solution.^{6c}

Cuprammonium solution, because of its rapid solvent action upon cellulose and the fact that it does not degrade the cellulose if proper precautions are observed, is the most suitable solvent for any cellulose preparation the viscosity of which is to be determined.

Instead of copper-tetraammino hydroxide, copper-ethylenediamine hydroxide may be used. The formula of the copper-ethylenediamine cellulose, which has been isolated,⁷ is $[\text{C}_{12}\text{H}_{16}\text{O}_{10}\text{Cu}][\text{Cu}(\text{En})_2]$ in which "En" represents ethylenediamine. In the copper-tetraammino compound the two ethylenediamine molecules are replaced by four ammonia molecules. Both compounds form complex metal salts. With sodium hydroxide, for example, a compound of the formula $[\text{C}_{12}\text{H}_{16}\text{O}_{10}\text{Cu}]\text{Na}_2$ is obtained.^{7a} The corresponding compounds are formed with the alkaline earths and with thallium nitrate.⁸

⁶ Hess, "Die Chemie der Cellulose," Akad. Verlags-Ges., Leipzig (1928), p. 306.

^{6a} Hess and Wittelsbach, *Z. Elektrochem.*, **26**, 232 (1920).

^{6b} Scheller, *Melliand Textilber.*, **16**, 787 (1935); Bancroft, paper presented before the American Chemical Society meeting at Pittsburgh, September, 1936.

^{6c} Heuser, "Lehrbuch der Cellulosechemie," 3rd ed., Borntraeger, Berlin (1927), p. 176.

⁷ Traube, *Ber.*, **63**, 2083 (1930).

^{7a} Hess and Messmer, *Ber.*, **55**, 2432 (1922); Heuser, *Papier-Fabr.*, **25**, 238 (1927).

⁸ Traube and Funk, *Ber.*, **69**, 1476 (1936).

The cuprammonium solution of cellulose also serves an important commercial purpose, namely, the manufacture of artificial silk ("copper silk," "cuprammon rayon"); the solution is forced through fine nozzles into dilute sulfuric acid and the cellulose is regenerated in the form of a thread of cellulose hydrate.

Cellulose may also be dissolved in a number of electrolytes, particularly those which exert a pronounced swelling effect upon it, as, for example, the thiocyanates. Usually an elevated temperature is required to achieve dissolution and the cellulose is more or less degraded. Recently some mineral acids (particularly phosphoric acid) have been described as good actual solvents for cellulose, when used with proper precautions.⁹ The use of quaternary ammonium bases as solvents has been mentioned before.^{4c}

CELLULOSE ESTERS

Just as an alcohol undergoes esterification with an acid in the presence of a dehydrating medium or by the action of an acid chloride, so cellulose may be converted into esters. Since, as will be seen later, of the five available hydroxyls in each glucose unit two are involved in the linkings with the neighboring glucose anhydrides, there are only three hydroxyls in cellulose which may be esterified. It has not always been possible to convert all three hydroxyls into ester groups, and esterification seems to arrest itself at two hydroxyls and sometimes even at one. This is due to the inactivity of the cellulose and, consequently, to the slowness with which most cellulose reactions proceed, which may be explained partly by assuming that in the bundles of glucose anhydride chains only a part of the hydroxyls are exposed on the surface, another inner part being less easily accessible so that means are needed to open the path for the agents to react. However, the three available hydroxyls often behave as if they are not equivalent. To facilitate access of the agents it suffices in many cases to bring about, with strong caustic soda solution, strong acids, or other means, a marked swelling, to which cellulose, as has been seen, yields easily. In other cases, it is even necessary to bring about a shortening of the long-chain molecule which, to a greater or lesser extent, may already have occurred owing to the nature of the dehydrating medium chosen, as for example concentrated sulfuric acid, particularly at elevated temperatures. The result of esterification, then, is a mixture of molecules of greater and smaller chain length, among which even oligosaccharides, cellobiose, and glucose may be found. All are esterified to a varying degree, and cumbersome

⁹ cf Ekenstam, *Ber.*, **69**, 549, 553 (1936).

methods of fractionating have to be resorted to in order to separate the mixture. For commercial purposes this procedure is omitted, and the mixture is used as such. However, if fiber structure and a certain physical strength are retained on esterification, it may be safely assumed that the reaction has proceeded without substantial degradation. On the other hand, the loss of fiber structure during esterification does not necessarily mean that chemical degradation has occurred. The cellulose fiber or the derivative formed may merely go into solution.

The cellulose esters are soluble in organic solvents, each ester having one (or more) most suitable solvent or mixture of solvents. The extent to which the esters dissolve is used as an indication of the completeness of esterification. It also serves to distinguish between di- and triesters. The viscosity of the solution is dependent upon the extent of degradation which the cellulose has undergone on esterification, low viscosity indicating a far-reaching degradation, and *vice versa*. The cellulose esters, like aliphatic esters, yield to saponification, whereupon cellulose and acid are regenerated. Sometimes it is possible to regenerate the cellulose without further degradation, and an investigation of the regenerate (e.g., by determining its viscosity in cuprammonium solution) shows how much degradation the cellulose has suffered during esterification.

The cellulose esters are of great technical interest, particularly the nitrate and the acetate.

Cellulose nitrate (nitrocellulose, nitric acid ester), $C_6H_7O_2(ONO_2)_3$, which theoretically requires a nitrogen content of 14.17 per cent, may be obtained by treating cellulose with a mixture of nitric and sulfuric or other mineral acids containing a certain percentage of water, at a temperature preferably not higher than 40°. The process of nitration seldom takes longer than an hour depending upon the concentration of the acids used, the proportion of nitric to sulfuric acid, and the temperature. Of the factors governing the process, the water content of the acid mixture is of predominating influence upon the characteristics of the nitrocellulose, particularly upon its nitrogen content and its solubility in certain solvents, such as ether-alcohol mixture. The use of higher temperature would be detrimental, considering the fact that the acid mixture not only brings about esterification but at the same time exerts a certain hydrolyzing and oxidizing effect upon cellulose, as well as a saponifying effect upon the ester. The results of such undesirable side reactions are impurities which have to be removed in order to obtain the pure ester. But even under normal temperature conditions (usually $30^\circ \pm 5^\circ$), smaller or larger amounts of impurities are formed, depending upon the cellulose raw material used. Impurities are removed by washing the nitrate with water and then boiling it with

water for several hours ("stabilization"). Drying has to be carried out with care since the nitrate is easily inflammable and, if detonated by a blow or by means of mercury fulminate, highly explosive. The only products of this detonation, besides water vapor, are gases—chiefly nitrogen, hydrogen, and carbon dioxide. It should be mentioned here that, because of the extreme hazards, drying of nitrocellulose is hardly ever practiced commercially. Instead it is handled wetted with water, or if desired, the water may be replaced by alcohol or some other suitable non-solvent for nitrocellulose.

The presence of sulfuric acid in the nitration acid is necessary to carry the reaction through within a reasonable time; without it, days are required for completion. Sulfuric acid may be replaced by phosphoric acid¹⁰ or by glacial acetic acid,¹¹ phosphoric acid leading to a trinitrate of the theoretical nitrogen content, 14.17 per cent, scarcely obtainable with the usual nitration acid mixture.

More recently it has been found possible to nitrate cellulose to a nitrogen content of 11–13 per cent within 18 to 48 hours at 20° by means of nitric acid fumes without the presence of a dehydrating agent except the nitric acid itself. The long time required tends to degrade the cellulose, which degradation is indicated by a decrease in the viscosity of the nitrocellulose solution in acetone, butyl acetate, or the like.¹² It is also claimed that nitric acid anhydride dissolved in carbon tetrachloride yields a pure trinitrate.¹³

With nitric acid of lower concentration (68.6 per cent) than that required for nitration (75–77 per cent), cellulose forms an addition compound which, because of its discoverer, is called "Knecht's compound." Its formula is probably $C_6H_{10}O_5 \cdot HNO_3 \cdot H_2O$, which would indicate that it is an addition compound of cellulose and the monohydrate of nitric acid. Its first appearance, with 62 per cent nitric acid, may be observed by x-ray photography.¹⁴ This also allows a following of the nitration process and recognition of it as a gradually proceeding esterification of the hydroxyl groups. But not until a nitrogen content of 12.5 per cent has been reached can a characteristic nitrocellulose fiber diagram be obtained.¹⁵ This shows that the hydroxyls of the cellulose are attacked in an irregular way—that is, the esterified groups are distributed unevenly in the micellae and probably also within the single chains, which means that mixtures result, so that the products termed

¹⁰ Berl and Rueff, *Cellulosechem.*, **14**, 115 (1933).

¹¹ Trogus, *Ber.*, **64**, 405 (1931).

¹² Rogovin and Tichonov, *Cellulosechem.*, **15**, 102 (1934).

¹³ Dalmon, Chédin, and Brissaud, *Compt. rend.*, **201**, 664 (1935).

¹⁴ Trogus, *Cellulosechem.*, **15**, 104 (1934).

¹⁵ Mathieu, *Compt. rend.*, **200**, 143 (1933).

mono- and dinitrates according to their nitrogen content cannot be regarded as homogeneous chemical individuals. The trinitrate alone gives a sharp and clearly patterned diagram. On rapid nitration it may happen that in some of the chains all three hydroxyls are nitrated simultaneously while in others only two or one are esterified. This produces mixed diagrams. It therefore appears doubtful whether nitration leads to any state of equilibrium before all available hydroxyls are nitrated.

It is possible that Knecht's compound, which easily separates into its components when treated with water, forms as an intermediate during nitration. The sulfuric acid ester of cellulose^{15a} which may form under certain conditions of nitration may also be regarded as an intermediate; it too is rather unstable.

The nitric acid ester may be saponified by means of strong sulfuric acid. The inorganic group is quantitatively regenerated as nitric acid, while the cellulose is largely degraded chemically. Such degradation also occurs with most of the other methods in which the nitrogen may be liberated as such, or in the form of nitric acid, nitric oxide, or other nitrogen compounds.

By saponification with alkali alone, whereby all nitrogen is converted into nitrite, the cellulose becomes oxidized. However, with potassium or other hydrosulfides which also convert all the nitrogen into nitrite, cellulose emerges with only slight signs of degradation.

Cellulose Acetate. Of the esters which cellulose forms with organic acids the acetate (acetylcellulose) is the most important; like the several modifications of the nitrate, the products of varying acetyl content have found a rather wide field of commercial use. Cellulose acetate may be obtained on treatment of cellulose with acetic anhydride (dissolved in acetic acid) in the presence of agents which exert a marked swelling action upon cellulose. For the sake of convenience these agents are termed "catalyzers." Sulfuric acid, sulfuryl chloride, and, less frequently, zinc chloride, are used. Simultaneously these agents exert a certain hydrolyzing effect upon cellulose, depending essentially upon the temperature. Under the influence of the reagents cellulose loses its fibrous structure and passes into the state of a thick, viscous paste. After acetylation is completed, the paste is poured into water whereby the water-soluble products of degradation are removed and the acetate is obtained in the form of white flocks. It is further purified by boiling in water.

Esterification, like nitration, proceeds only slowly so that after certain intervals step products are obtained which, again, are nothing

^{15a} Reference 6c, p. 54; Traube, Blaser, and Grunert, *Ber.*, **61**, 754 (1928).

but mixtures of partly acetylated chains with others still intact or more or less degraded. The further esterification proceeds—i.e., the more hydroxyls react, the more uniform the product of reaction becomes, until the triacetate is reached. Thus only the triacetate, after esters of degraded chains have been removed, may be regarded as uniform.^{15b} It has very nearly the acetyl content which is theoretically required for $C_6H_7O_2(OCOCH_3)_3$, namely, 44.8 per cent (62.5 per cent combined acetic acid).

The triacetate is soluble in chloroform and insoluble in acetone; it is resistant to boiling water and to temperatures up to 125°; it begins to melt at about 250°. The solution in chloroform and in other solvents, such as tetrachloroethane and pyridine, shows levorotation. With progressive degradation of the cellulose during acetylation (acetolysis) the levorotation inverts to dextrorotation.

On careful acetylation, particularly by diluting the acetic anhydride-sulfuric acid mixture with benzene or carbon tetrachloride (i.e., solvents in which the acetate is insoluble), the fibrous structure of the original cellulose is maintained. Thus a product suitable for making x-ray fiber diagrams is obtained, and as in nitration, acetylation may be followed and well recognized as a topochemical reaction.^{15c} Another way of following the process also adaptable to nitration¹⁶ is to observe the change in double refraction of the fibers undergoing acetylation.

From the foregoing it will be readily understood that in acetylation also it is practically impossible to obtain the lower steps (the di- and the monoacetate) in pure form. On attempting to adjust esterification in order to arrive at the lower steps, only a mixture results whose acetyl content, if found to be that of the diacetate (48.8 per cent combined acetic acid), is merely misleading. The same holds true if one attempts to obtain the lower stages by partial saponification with mineral or certain organic acids. This method is of commercial importance, since in such a partial saponification, e.g., to a product whose acetyl content ranges between that of a tri- and a diacetate (37–42 per cent, corresponding to 51–58.6 per cent of combined acetic acid), certain changes in physical structure seem to be involved which are essential for the production of films, for instance, of sufficient elasticity and physical strength.^{16a} The last-named properties are directly related to the viscosity of the solution. While the triacetate is soluble in chloroform, the partially saponified product is soluble in acetone, which serves as

^{15b} Ost, *Z. angew. Chem.*, **32**, 66, 76, 82 (1919).

^{15c} Reference 4e, p. 274.

¹⁶ Karamaru, *Helv. Chim. Acta*, **17**, 1429 (1934).

^{16a} See, however, Elöd and Schrodtt, *Z. angew. Chem.*, **44**, 933 (1931).

the basis for commercial utilization. Reacetylation yields a uniform triacetate.

Complete saponification, which may be brought about by means of acids or alkalis¹⁷ regenerates the cellulose in a more or less degraded form, depending upon the agent used and the conditions chosen. If in both acetylation and saponification mild processes are employed, the regenerated cellulose appears scarcely degraded. This is supported by the x-ray pattern of samples thus prepared, which is the same as the pattern of the "mercerized" cellulose regenerated from alkali cellulose.

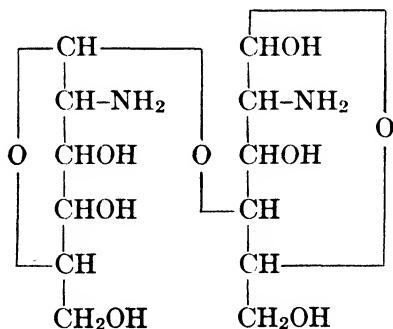
Cellulose undergoes esterification with a great number of other acids. The sulfuric and phosphoric acid esters play a certain part as intermediates during acetylation if such acids are used as "catalyzers."

Of the organic acids the following have been more or less successfully used for esterification of cellulose: formic, chloroacetic, carbonic, propionic, butyric, stearic, lauric, palmitic, caprylic, oxalic; also aromatic acids such as benzoic, phthalic, cinnamic,^{17a} and a number of sulfonic acids, particularly *p*-toluenesulfonic acid.

Aminocellulose. The ester of *p*-toluenesulfonic acid, $C_6H_5O_4OSO_2C_6H_4CH_3$, is obtained by the action of the chloride of *p*-toluenesulfonic acid on alkali cellulose. Under the influence of ammonia the toluenesulfonyl group is said to be removed and replaced by an amino group, resulting in aminocellulose.

It has been reported that amines, such as aniline, perform the substitution reaction on the *p*-toluenesulfonate of cellulose.¹⁸

Aminocellulose may also be termed polymerized aminoglucose anhydride (p. 1484), and as such it bears a certain resemblance to chitin or



¹⁷ Zemplén, *Ber.*, **69**, 1827 (1936).

^{17a} Reference 6c, p. 71; Hess, "Die Chemie der Cellulose," p. 428.

¹⁸ Karrer and Wehrli, *Z. angew. Chem.*, **39**, 1509 (1926); Sakurada, *J. Soc. Chem. Ind. Japan*, supplemental binding, **32**, 11B (1929); Hess and Ljubitsch, *Ann.*, **507**, 62 (1933). According to information recently received, the introduction of amino groups into cellulose has not been confirmed by other investigators. However, amines such as methylamine and diethylamine seem to react if the cellulose is in an alkaline solution.

its derivative which results from acetolysis (acetylation and subsequent hydrolysis) of lobster shells and which has been identified as chitobiose, (p. 1547) a diglucosamine.^{18a} The similarity of chitin and cellulose is also revealed by x-ray studies on chitin, chitosan, and derivatives.¹⁹

Finally, so-called mixed esters may be prepared, as nitrocellulose acetates, nitrocellulose benzoates, and so forth.^{19a}

Cellulose Xanthate. Of special interest, both from a scientific and a commercial point of view, is cellulose xanthate—that is, the dithiocarbonic acid ester of cellulose (also, but not quite correctly, called the xanthogenic acid ester of cellulose) already mentioned in connection with the discussion of alkali cellulose. It is formed when alkali cellulose is subjected to treatment with carbon disulfide, the former going through a state of high swelling, being dissolved eventually to a highly viscous, orange-colored paste, the orange color arising from the sodium trithiocarbonate formed as a by-product.

As already mentioned, the reaction has been compared with that of carbon disulfide on sodium alcoholate, wherein the sodium salt of the dithiocarbonic acid ester (xanthogenic acid) is formed. It is interesting to note that carbon disulfide alone does not react with cellulose. This supports the concept of alkali cellulose as a chemical compound. As a matter of fact, the xanthate reaction is accomplished only if, in the preparation of the alkali cellulose, a sodium hydroxide solution of such concentration is used that the compound $(C_6H_{10}O_5)_2 \cdot NaOH$ can be formed. Dimethylcellulose (p. 1552), which does not form an alkali compound, is also unable to form a xanthate; a lower methylated product (corresponding to a monomethylcellulose), however, undergoes the xanthate reaction since it forms an alkali cellulose.^{19a} The cellulose xanthate may be expressed by the formula $NaS-CS-O(C_6H_9O_4)_2OH$, which indicates that two glucose anhydride units combine with one molecule of carbon disulfide and one atom of sodium.

The nature of the alkali cellulose prepared with sodium hydroxide and regarded as a chemical compound in which two units of glucose anhydride combine with one molecule of sodium hydroxide seems to limit the xanthate reaction to one case only. In other words, since cellulose combines only with one molecule of sodium hydroxide, only a monoxanthate may be expected. If this is so, the xanthate reaction would appear to be independent of the hydroxyls available (which has been observed in some cases with alcohols). Until recently the various

^{18a} Bergmann, Zervas, and Silberkweit, *Ber.*, **64**, 2436 (1931).

¹⁹ Clark and Smith, *J. Phys. Chem.*, **40**, 863 (1936).

^{19a} Heuser and Schuster, *Cellulosechem.*, **7**, 17 (1926).

endeavors to introduce more than one dithiocarbonic acid group have not been successful (not even in cellulose whose hydroxyls had been made more reactive by acetylation).^{19b} Neither has it been possible to obtain a xanthate with alkali celluloses prepared with sodium hydroxide solutions of much higher concentration than 18 per cent.²⁰ On the other hand, the actual cellulose alcoholate in which the three hydrogens of the three hydroxyls are replaced by three sodium atoms (see above) ought to yield a trixanthate. Recently Lieser and Leckzyk²¹ have described a trixanthate of cellulose. Upon replacing sodium hydroxide by tetraethylammonium hydroxide, in which cellulose dissolves, the action of carbon disulfide leads to xanthation of all three available hydroxyls of cellulose. The tri-tetraethylammonium xanthate of cellulose thus obtained is soluble in methyl alcohol, water, and acetone.

This investigation was preceded by studies of the xanthation of simple sugars.²² α -Methylglucoside under the influence of barium hydroxide and carbon disulfide yields a monoxanthate in crystalline form, together with small amounts of di- and trixanthates. Better yields of the polyxanthates are obtained from β -phenylglucoside, using tetraethylammonium hydroxide as the base. Thus a tetramethylxanthate of the β -phenylglucoside could be obtained in crystalline form.

That it is the hydroxyl in the 2-position of the glucose units in cellulose which undergoes the xanthate reaction is indicated by methylation studies. With diazomethane the xanthate suffers hydrolysis, and a methylated product is obtained in which two glucose anhydride units are combined with one methyl group ($C_6H_{10}O_5 \cdot C_6H_9O_4 \cdot OCH_3$; "mono-methyl-dicellulose"). On hydrolysis, glucose and monomethylglucose with the methoxyl group in the 2-position are obtained.²³

The normal cellulose xanthate is soluble in water and dilute alkalis. It may be precipitated from its solution by means of alcohols or salts, and also by very dilute organic acids, such as acetic. With stronger acids the xanthate decomposes into its components: cellulose, carbon disulfide, and sodium hydroxide. This same reaction takes place under the influence of the carbonic acid of the air. The cellulose emerges from its state in solution chemically unchanged, showing the behavior and reactions of "mercerized" cellulose (cellulose hydrate).

The xanthate solution also decomposes without the action of acids when allowed to stand in a hydrogen or nitrogen atmosphere or when

^{19b} Heuser and Schuster, *ibid.*, **7**, 28 (1926).

²⁰ Atsuki und Kuvahawa, *Cellulose Ind.*, **1**, 47 (1931).

²¹ Lieser and Leckzyk, *Ann.*, **522**, 56 (1936).

²² Lieser and collaborators, *Ann.*, **495**, 235 (1932); **511**, 121, 128 (1934); **519**, 279 (1935); **522**, 48 (1936).

²³ Lieser, *Ann.*, **470**, 104 (1929); **483**, 132 (1930).

being dialyzed with water. The process is very slow, however, requiring three to four days for its completion. The spontaneous decomposition (distinctly accelerated in air) is termed the "ripening" of the viscose. It consists chemically of a gradual saponification of the ester under the influence of the aqueous alkali present, so that the xanthates isolated during this process become richer in cellulose until all sulfur and sodium are split off and pure cellulose results.

The other products are chiefly sodium trithiocarbonate (Na_2CS_3) and sodium carbonate, the former being partly decomposed in the presence of water to sodium carbonate and sulfide. On decomposition of the viscose with mineral acids the by-products suffer instantaneous cleavage into the respective sodium salt, carbon disulfide, and hydrogen sulfide, while the xanthate decomposes into the sodium salt, cellulose, and carbon disulfide. Determination of the amounts of carbon disulfide obtainable from the xanthates isolated during ripening may be used for following the ripening process of the xanthate. In commercial viscose, determination of the total amounts of carbon disulfide and hydrogen sulfide present during ripening serves the same purpose.

Physically, the ripening process is most essentially characterized by a change in viscosity of the viscose. The solution first becomes thinner, until the coarser particles are dispersed to the smallest possible size, whereafter the viscosity slowly increases, owing to the salting-out effect of the by-products formed from the decomposing xanthate. The last state is characterized by a more rapid increase in viscosity, which indicates that decomposition is nearing its final stage. The cellulose eventually regenerated shows about the same viscosity in cuprammonium solution as that regenerated at the beginning of the ripening process, proving that the cellulose constituent is not degraded during the ripening process.^{23a}

This is in contrast to the "aging" process (see under "alkali cellulose") during which degradation, physical and chemical, takes place, and during which the viscosity of the regenerated cellulose in cuprammonium solution decreases. It is this state of degradation which determines the initial and the final viscosity of the viscose solution and, consequently, the degree of degradation of the cellulose regenerated from the viscose.

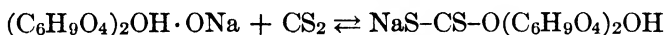
If oxygen, in the form of air or other oxidants, is given the opportunity to penetrate the viscose thoroughly, the effect manifests itself chiefly in a faster rate of viscosity decrease during ripening.²⁴ With increased admittance of oxygen the orange-colored viscose becomes

^{23a} Heuser and Schuster, *Cellulosechem.*, **7**, 40 (1926).

²⁴ Lottermoser and Schwarz, *Z. angew. Chem.*, **43**, 16 (1930).

colorless; the trithiocarbonate causing the reddish orange color is oxidized, as are the other sulfur compounds, while the cellulose suffers but little.²⁵

Most of the authors who have interested themselves in the mechanism of the xanthate reaction are inclined to believe that a chemical reaction takes place and that the xanthate is a chemical compound, i.e., one in which the three components are found in stoichiometric proportions. The fact that the xanthate reaction takes place with less carbon disulfide than is stoichiometrically required may be explained by assuming that in the viscose the xanthate is in equilibrium with the alkali cellulose. This may be expressed by the equation,

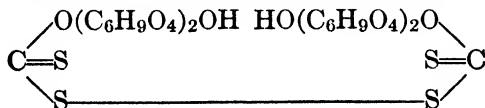


in which the alkali cellulose is assumed to react in the form of its alcoholate. There seems to be no doubt that, immediately after the action of carbon disulfide on alkali cellulose, a xanthate of the above composition is formed. This is further supported by the fact that the xanthate reacts with diethylchloroacetamide $[\text{ClCH}_2\text{CON}(\text{C}_2\text{H}_5)_2]$ to form a

homogeneous derivative, $-\text{C}-\text{O}-\text{C} \begin{smallmatrix} \text{S} \\ \parallel \end{smallmatrix} \text{S}-\text{CH}_2\text{CON}(\text{C}_2\text{H}_5)_2$, in which

the nitrogen content is in direct proportion to the cellulose content.^{25a} However, the ripening process, judging from x-ray investigations,²⁶ does not seem to lead to intermediates in which there is a distinct stoichiometric relationship between the constituents sulfur, sodium, and cellulose, as has been claimed by previous investigators. These intermediates appear to be mixtures, and the mechanism seems to consist merely in gradual saponification (hydrolysis) as mentioned above—that is, without it being possible to isolate chemically homogeneous intermediates.

The aqueous solution of the xanthate consumes iodine to form sodium iodide. This reaction serves to determine the sodium content of the xanthate. It is claimed that a dicellulose dithiocarbonate is formed simultaneously according to the equation:



²⁵ Lottermoser and Schwarz, *Kolloid-Beihefte*, **42**, 408 (1935).

^{25a} Fink, Stahn, and Matthes, *Angew. Chem.*, **47**, 602 (1934).

²⁶ Schramek and Küttner, *Kolloid-Beihefte*, **42**, 221 (1935).

CELLULOSE ETHERS

Cellulose ethers may be obtained in the same way as aliphatic ethers, for example, by the action of alkyl halides or alkyl esters of inorganic acids upon alkali cellulose. The latter way is the usual one. What has been said with regard to the slow reaction in the formation of esters holds true also in the conversion of cellulose into its ethers. It is very likely that in most reactions the mono- and diethers must be regarded as mixtures and that only the triether represents a homogeneous product. More recently, as a result of fractionation, it has been claimed that even trimethylcellulose is a mixture.²⁷

It appears that cellulose is even more resistant to etherification than to esterification, and the reaction appears to proceed more easily the more the cellulose has been physically and chemically degraded. Thus cellulose regenerated from solutions or from processes of oxidation or from the action of acids can be converted into ethers within a shorter time than untreated "native" cellulose.

Methyl and Ethyl Ethers. The most important cellulose ether is methylcellulose since it, as will be seen later, has greatly assisted the endeavors to clear up the chemical constitution of cellulose. Commercially, the ethyl ether may be regarded as the most important.

The usual way of preparing methylcellulose consists in allowing dimethyl sulfate to react on alkali cellulose at a temperature of about 50°. ^{27a} The reaction is exothermic, but the heat developed does not suffice to accelerate the reaction to any great extent. Usually more than one methylation is required to reach the trimethylate (theoretically 45.57 per cent methoxyl), particularly with native cellulose. ^{27b} Methylation may be facilitated if the hydroxyls of the cellulose are "activated" by acetylation. It appears feasible first to methylate to the dimethyl stage, which is comparatively easily reached, then to acetylate to dimethylmonoacetylcellulose, and then methylate again, whereupon the acetyl is saponified and replaced by the methyl group. The trimethylate thus obtained contains 45.42 per cent methoxyl. ²⁸

Of interest is the behavior of methylcellulose to water. ²⁹ Products with $1\frac{1}{2}$ to 2 methoxyls per glucose unit are soluble in cold water. On heating they are precipitated, and on cooling they go into solution again.

²⁷ Hess and collaborators, *Cellulosechem.*, **16**, 78 (1935).

^{27a} Denham and Woodhouse, *J. Chem. Soc.*, **103**, 1735 (1913); **105**, 2357 (1914); **111**, 244 (1917); **119**, 81 (1921).

^{27b} Heuser and von Neuenstein, *Cellulosechem.*, **3**, 92 (1922); Irvine and Hirst, *J. Chem. Soc.*, **123**, 529 (1923).

²⁸ Heuser and Hiemer, *Cellulosechem.*, **6**, 101 (1925).

²⁹ Traill, *J. Soc. Chem. Ind.*, **53**, T. 337 (1934).

Very likely hydrates which suffer cleavage on heating are formed. The stability of the hydrates is greater the lower the temperature of the water. It was found that the temperature at which the precipitate obtained on heating the ice-cooled solution redissolves, is directly related to the degree of degradation of the cellulose preparation used: the greater the degradation, the less is it necessary to cool the precipitate in order to bring about re-solution. Thus the "temperature of hydration" may be used to express the degree of degradation of the cellulose.²⁸ Completely methylated cellulose is insoluble in water. The ethers of varying methoxyl content are soluble in pyridine, chloroform, tetrachloroethane, etc., and show different levorotational values in the various solutions.

The methyl groups may be regenerated as from aliphatic ethers by allowing hydrogen iodide to act on the ether. The regenerated cellulose is extensively degraded as a result of the violent character of this reaction.

It is evident from x-ray analysis, as well as from hydrolysis and acetolysis studies, that the intermediates are mixtures of the three stages of methylation. On hydrolysis, dimethylcellulose usually yields mono-, di-, and trimethylglucose. However, it is claimed that an ethylcellulose preparation with $2\frac{1}{2}$ ethoxyl groups for each $C_6H_{10}O_5$ unit, on acetolysis with acetyl bromide and hydrogen bromide, gave only bromodiacetyldiethylglucose.³⁰ Trimethylcellulose on hydrolysis yields 2,3,6-trimethylglucose, which shows that the hydroxyls in positions one, four, and five of the glucose anhydrides in cellulose are blocked. Besides, a small amount of tetramethylglucose is obtained.^{30a} The importance of this discovery for determining the chemical constitution of cellulose will be explained later. Again, on acetolysis the corresponding acetylated alkyl glucoses are formed; e.g., triethylcellulose yields 1,4-diacetyl-2,3,6-triethylglucose.

Methylene ethers (acetals) of cellulose have also been synthesized, by allowing formaldehyde or aliphatic methylene ethers or methylal to react upon alkali cellulose.

The reaction of formaldehyde with cellulose has its parallel in the action of this aldehyde upon *d*-glucose, which leads to methyleneglucose (Tollens), the mechanism being that of acetal formation. Since acetals are rather sensitive to water, a dehydrating medium must be used. With acetic anhydride and the *n*-propyl or isopropyl acetal of formalde-

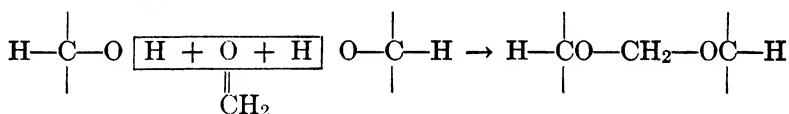
³⁰ Hess, Trogus, *et al.*, *Ann.*, **506**, 260 (1933).

^{30a} See reference 27a; also, Haworth and Leitch, *J. Chem. Soc.*, **113**, 191 (1918); Irvine and Hirst, *ibid.*, **123**, 529 (1923); Hess and Weltzien, *Ann.*, **442**, 46 (1925); Hess, *Angew. Chem.*, **49**, 841 (1936); Hess and Neumann, *Ber.*, **70**, 710, 721, 728 (1937).

hyde, a combination which liberates formaldehyde easily, 8 per cent of methylene radical can be introduced into cellulose, which indicates that one methylene group is in combination with two hydroxyls of one $C_6H_{10}O_5$ unit.³¹ Under the influence of the acetic anhydride, partial acetylation takes place, but with dilute alkali the acetylated hydroxyls are regenerated and an acetyl-free methylene ether of cellulose is obtained.

With dichlorodimethyl sulfate, $(ClCH_2O)_2SO_2$, or similar esters which contain the $-CH_2O$ group, it is possible to increase the methylene content considerably (17.2 per cent CH_2O),³² whereas by the action of methylal $[CH_2(OCH_3)_2]$ only about 7 per cent of CH_2O is obtained.³³

Since for steric reasons it is rather improbable that "methylenation" takes place on two neighboring $-CHOH$ groups of the glucose anhydrides of one and the same chain,³⁴ for example, on those in positions two and three lying in different planes, it is assumed rather that two hydroxyls of neighboring chains join in this reaction, possibly according to the following scheme:



This concept of the reaction mechanism may explain why only a small amount of methylene radical in the cellulose suffices to bring about interesting physical changes which, with increasing percentages of methylene content, become very obvious and manifest themselves in a decrease in the swelling ability in water even to zero, the methylene derivative becoming so brittle that it may be pulverized. Moreover the products lose all the reactivity of the original cellulose: they become more and more insoluble in cuprammonium solution, they do not react with alkalis to form alkali cellulose, and they do not undergo the xanthate reaction. The methylene linking between neighboring chains may well explain the loss of elasticity of the micellar system with increasing "methylenation."

Glycolic Acid Ether. In the action of monochloroacetic acid on alkali cellulose the chlorine reacts with the alkali metal to form sodium chloride and the glycolic acid ether of cellulose, which may be formulated as $C_6H_7O_2(OCH_2CO_2H)_3$, results. It is insoluble in water but capable of forming salts. The sodium salt is soluble in water and forms

³¹ Schenk, *Helv. Chim. Acta*, **15**, 1088 (1932).

³² F. Wood, *J. Soc. Chem. Ind.*, **50**, T. 411 (1931).

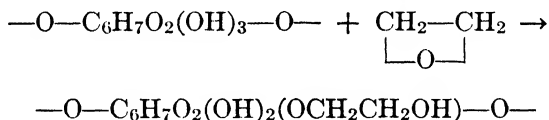
³³ Schorigin and Rymaschewskaja, *Cellulosechem.*, **14**, 81 (1933).

³⁴ Meunier and Gyot, *Compt. rend.*, **188**, 506 (1929).

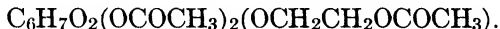
a viscous solution. The ether is insoluble in organic solvents such as alcohol, ether, benzene, and acetone. The formulation of an acid ether of cellulose appears justified since the action of phosphorus triiodide and water on the ether liberates glycolic acid ($\text{CH}_2\text{OH}-\text{CO}_2\text{H}$) and cellulose is regenerated. A small amount of acetic acid is also formed, which, however, may be due to reduction of part of the glycolic acid.^{3,4a}

Triphenylcarbinyl Ether of Cellulose. Apparently analogous to the action of triphenylchloromethane on alcohols and on certain sugars, such as α -methylglucoside, *d*-glucose, and *d*-galactose, is the reaction which leads to a triphenylcarbinyl ether of cellulose. Only one triphenylmethyl group seems to enter on one $C_6H_{10}O_5$ unit. The ether, $C_6H_9O_4-O-C(C_6H_5)_3$, dissolves in pyridine, chloroform, etc., to viscous colloidal solutions. It is very sensitive to acids, e.g., hydrochloric, and suffers cleavage to cellulose and triphenylmethyl chloride (or carbinol), respectively. The cellulose appears to have undergone scarcely any degradation during etherification.^{3,4b}

Glycolcellulose (Hydroxyethylcellulose). Ethylene oxide reacts with alkali cellulose to form so-called glycolcellulose or hydroxyethylcellulose, a product of possible commercial value. It is soluble in water and acetic acid, but insoluble in acetone, alcohol, and ether. Since all monosubstituted glycol ethers are soluble in water, it may be assumed that in hydroxyethylcellulose the ethylene oxide radical is present with a free hydroxyl group, $\text{—OCH}_2\text{CH}_2\text{OH}$. The mechanism of the reaction apparently is that of addition and may be illustrated thus:



Acetylation leads to a triacetate in which two acetyls occupy the two free hydroxyls of the glucose unit and the third acetyl has entered the hydroxyethylene radical. It may be formulated thus:³⁵



Benzylcellulose. One of the more recent cellulose derivatives, benzylcellulose, is obtained by the action of benzyl chloride on alkali cellulose. It seems to be difficult to introduce more than two benzyl

^{34a} Chowdhury, *Biochem. Z.*, **148**, 85 (1924); see, also, Barnett, *J. Soc. Chem. Ind.*, **40**, T. 253 (1921).

^{34b} Helferich and Koester, *Ber.*, **57**, 587 (1924); Helferich, Moog, and Jünger, *Ber.*, **58**, 872 (1925).

⁵⁵ Schorigin and Rymaschewskaja, *Ber.*, **66**, 1014 (1933).

groups per C₆-unit. The benzyl ether is exceedingly hydrophobic and for this reason is of special commercial value.³⁶

Besides the ethers described above there are a number of others not yet sufficiently investigated, of which, however, may be mentioned butyl-, propyl-, and allylcellulose.³⁷

THE OXIDATION OF CELLULOSE

For a long time many cellulose research workers fostered the belief that on oxidation the long-chain cellulose molecule is maintained, without the oxygen bridges between glucose anhydrides being ruptured. From inspection of the structural formula of Tollens or that of the modern conception (p. 1583) this appears quite possible. At first glance there seems to be no reason why the free alcohol groups in the 6-position of the glucose units could not be oxidized to aldehyde and further to carboxyl groups without breaking oxygen bridges. There are examples enough among the higher members of the sugars where the reaction takes place in this way, provided sufficient care is taken (this consists chiefly in the choice of a suitable oxidizing agent). Naturally, hydrolysis may occur if nitric acid is chosen as the oxidizing agent. Thus, cellobiose under these conditions yields the dibasic saccharic acid (p. 1410) and oxalic acid. With bromine water, however, the glycosidic linkage remains intact and the reducing group is attacked, cellobionic acid being the resulting product. A number of indications strengthen the belief that the oxygen bridges are maintained under certain conditions of oxidation.^{37a}

Early research workers such as Tollens and Vignon thought it possible that the glucose anhydrides on oxidation are converted into glucuronic acid groups (lactones) (p. 1448) still held together in a long-chain molecule (oxycellulose). Indeed, glucuronic acid, in the form of its cinchonine salt, has been isolated from the action of permanganate on cellulose dissolved in cuprammonium solution, with a yield of almost 10 per cent of the cellulose, and there is indication that the reaction product is still of a high-molecular nature until it is converted into the cinchonine salt, whereby cleavage may occur.³⁸ From this and other indications, it does not seem justified to deny completely, as some modern

³⁶ Gonfard, "Sur les propriétés de la benzylcellulose," Camus, Lyon (1933).

³⁷ Mienes, "Celluloseester und Celluloseäther unter besonderer Berücksichtigung der Benzylcellulose," Chem.-techn. Verl. Bodenbender, Berlin (1934). Lorand, paper presented before the American Chem. Soc. meeting at Pittsburgh, September, 1936.

^{37a} See Hudson, paper presented before the Cellulose Division of the American Chemical Society at Chapel Hill, N. C., April, 1937.

³⁸ Kalb and Falkenhäuser, *Ber.*, **60**, 2514 (1927); see, also, Heuser and Stöckigt, *Cellulosechem.*, **3**, 61 (1922).

cellulose chemists have done,³⁹ the hypothesis of oxidation without cleavage. On the other hand, it must be admitted that, as yet, it has not been possible to give satisfactory proof for the hypothesis; in other words, no one has yet been able to isolate and to identify unequivocally a product of oxidation which approaches the high molecular weight of cellulose itself. On careful oxidation a large part of the cellulose emerges unchanged, and only a small part shows signs of having been oxidized, this part indicating a rather low-molecular nature. On more violent oxidation the proportion which is being oxidized increases, but without revealing with certainty any unbroken molecule. This state of affairs will be more easily understood when it is considered how difficult it is to manipulate hydrolytic or acetolytic degradation processes on cellulose so as to arrive at degradation products containing more than two glucose anhydrides. The highest number which has been isolated so far is six, in the so-called "hexaose" (p. 1562). In other words, mild oxidation does not seem to lead anywhere, and violent oxidation breaks down the large molecule into fractions that are too small.

The difficulties of identification in oxidation and also the possibilities of being misled, are increased by the fact that most of the oxidative treatments in question lead to physical degradation of the cellulose, in part or entirely. That is to say, the cellulose after treatment loses its fibrous structure at a slight touch; it may easily be converted into a powder. This is why the technician who has to bleach textile fibers or wood pulp, etc., by means of oxidants such as bleaching powder, other hypochlorites, or peroxides, tries to direct the process so as to avoid the formation of oxycellulose, i.e., oxidation of any of the material except the non-cellulosic substances.

Physically degraded oxycellulose is partly or entirely soluble in dilute alkalis. This property has given rise to the assumption that oxycellulose is an acid, the alcoholic ($-\text{CH}_2\text{OH}$) groups having been converted into carboxyl groups.^{39a} However, if all the $-\text{CH}_2\text{OH}$ groups of the cellulose molecule had been oxidized there would be a greater number of characteristic and unequivocal indications than the mere solubility in alkalis.

This also holds true with regard to indications that have actually been observed on carefully oxidized cellulose, such as the liberation of carbon dioxide and furfural on distillation of oxycellulose preparations with dilute hydrochloric acid according to Tollens' method. The quantities of these two substances, which owe their formation to a cleavage

³⁹ Hess, "Die Chemie der Cellulose," p. 459.

^{39a} Schwalbe and Becker, *Ber.*, **54**, 545 (1921); Hibbert and Parsons, *Cellulosechem.*, **7**, 97 (1926).

of glucuronic acid with loss of water, certainly are greater than those obtained from untreated native cellulose but still much too small to account for more than a very limited number of carboxyl groups.^{39b} It therefore looks as if on oxidation (as usually carried out by allowing the oxidant to react on fibrous cellulose) only small amounts of reaction products form on the surface and these, under continued attack, suffer cleavage to lower-molecular products. If, however, cellulose in solution is oxidized, in which case excessive surface reactions are eliminated and the oxidant has access through the spaces between and inside the micellae to all molecules, the molecules may retain their original length, and on reprecipitation true oxycellulose may be obtained. The fact that a comparatively large yield of glucuronic acid has been obtained, and that it is colloidal in character (before conversion into its cinchonine salt), may be interpreted in the light of the conception advanced above.

Another indication of the possible mechanism of oxidation of cellulose is the golden yellow coloration which occurs when oxycellulose is heated in dilute caustic soda solution. This coloration is much more pronounced than that produced with untreated cellulose and is characteristic of the presence of aldehydic groups. In fact, the products of oxidation exert a pronounced reducing action on Fehling's solution or on a hypiodite solution.^{39c} These reactions and that with phenylhydrazine, which appears to be more than a mere adsorption on the increased surface of the oxycellulose, point in the direction of aldehydic functions having been developed, and it may be assumed that oxidation first leads to such groups before going further, and that the product of oxidation obviously contains both aldehyde and carboxyl groups. Attention may also be directed to the possibility that the mechanism of reaction, depending upon the means of oxidation chosen, may be that of dehydration to ketonic alcohols or diketonic alcohols, which would also exhibit a strong reducing power with Fehling's solution.³⁹

If, on oxidation of solid cellulose, physical degradation has not gone too far, it is possible to free the preparation of its aldehyde and carboxyl functions by means of extraction with dilute caustic soda solution. Thereby chemically unchanged cellulose is obtained as a residue, while the solution contains the low-molecular-weight products of oxidation. Depending upon the oxidants chosen for treatment, particularly whether they are of purely oxidizing or of both oxidizing and hydrolyzing nature, the products which may be extracted differ. With purely oxidizing

^{39b} Hibbert and Parsons, *J. Soc. Chem. Ind.*, **44**, T. 473 (1925); Heuser and Stöckigt, *Cellulosechem.*, **3**, 61 (1922).

^{39c} Willstätter and Schudel, *Ber.*, **51**, 780 (1918).

means glucuronic acid and a number of other acids not yet clearly identified result. The last stage before carbon dioxide and water are formed is manifested by the presence of oxalic acid. It is very probable that this acid originates from an intermediate, formic acid, with loss of hydrogen.

Oxidation of solid cellulose with bromine in the presence of calcium carbonate (in order to neutralize hydrogen bromide) results in oxycellulose from which a certain amount of the dibasic saccharic acid may be extracted by means of calcium carbonate.^{39d} This indicates that, besides oxidation of $-\text{CH}_2\text{OH}$ groups, glucosidic linkages have been broken and the reducing groups thus freed have been attacked. Saccharic acid is also obtained on oxidation of cellulose with hot concentrated nitric acid.

When the same oxycellulose is heated with calcium hydroxide solution, isosaccharinic acid and dihydroxybutyric acid may be isolated. It is of importance in this connection to note that unoxidized cellulose does not yield isosaccharinic acid on the same treatment, nor has isosaccharinic acid been obtained from glucose. This and the fact that it may be obtained from cellobiose would indicate that it owes its formation to the presence in the oxycellulose of a glucose group still glucosidically combined.⁴⁰ The other acid, dihydroxybutyric acid, is perhaps a product of further decomposition of isosaccharinic acid (p. 1516).

THE DEGRADATION OF CELLULOSE BY ACIDS

Besides oxidation, cellulose may be decomposed (1) by hydrolytic processes, (2) by thermal processes, and (3) by biological processes.

Hydrolysis. With mineral acids (less completely with organic acids), hydrolysis leads to glucose through a number of intermediate stages; on acetolysis the hydroxyls of the degradation products appear partly or entirely acetylated. Here again, as with other treatments, cellulose reacts slowly and irregularly. Degradation starts on the surface of the fiber and may lead comparatively easily to lower-molecular units and even to the end product. Deeper layers, on the other hand, are attacked to a lesser degree, and in consequence interruption of the process after a certain time yields a variety of degradation products, from almost untouched chains down to the monomeric glucose.

Hydrocellulose. Although treatment with concentrated acids leads to complete hydrolysis, the intermediate products of degradation can be obtained to a certain extent by the use of dilute acids. On heating

^{39d} v. Faber and Tollens, *Ber.*, **32**, 2592 (1899).

⁴⁰ Pringsheim, *Cellulosechem.*, **2**, 61 (1921); *Z. angew. Chem.*, **35**, 348 (1922).

cellulose fiber with dilute hydrochloric acid a product is obtained whose appearance and behavior resemble oxycellulose. Like the latter, cellulose emerges from the treatment as weakened fiber which may easily be rubbed to powder between the fingers. It is termed "hydrocellulose" and for many years has been regarded as the first homogeneous product of hydrolysis.^{40a} The term is derived from the assumption that it is cellulose to which one molecule of water is chemically attached. Considering cellulose as a chain of anhydrides linked together by means of oxygen bridges, there is no reason to regard hydrocellulose as a homogeneous cellulose derivative as may be true of oxycellulose, for it may be assumed that on oxidation certain groups of the still untouched chain are essentially changed. In the formation of hydrocellulose the only change conceivable is a shortening of the long chains. Under the hydrolyzing effect of the acid, a number of oxygen bridges may break up, resulting in a mixture of saccharides of lower molecular weight and pronounced reducing power, with chains still of considerable length.

This concept of hydrocellulose^{40b} is derived from its behavior with dilute alkalis. On heating with a 4-6 per cent sodium hydroxide solution the raw hydrocellulose preparation may be divided into two components. The filtrate shows pronounced reducing power to Fehling's or sodium hypiodite solution, and it contains the lower degradation products; the residue after sufficient purification shows little if any reducing power, and, on being compared with native cellulose, does not reveal any chemical change. It is soluble in sodium hydroxide solution of a certain higher concentration (preferably 8 per cent),^{40c} which may be explained as resulting from the shortening of the chains. This state of the preparation which apparently cannot be identified as a chemical change, and which therefore appears as a state of physical degradation only, is also reflected in the viscosity of its solution. This is found considerably decreased compared with that of the starting material. The purified alkali-soluble hydrocellulose has more recently also been termed "cellulose A" for the sake of convenience.^{40c}

The amount of low-molecular degradation products in hydrocellulose is very small. It is possible to convert up to 99 per cent of the original cellulose into purified alkali-soluble cellulose. Organic acids such as

^{40a} See, for example, Schwalbe, "Chemie der Cellulose," Borntraeger, Berlin (1911); see, also, Heuser and Stöckigt, *Cellulosechem.*, **3**, 61 (1922); Heuser and Jayme, *Ber.*, **56**, 1242 (1922).

^{40b} Reference 6c, p. 157; see, also, Heuser and Hiemer, *Z. Elektrochem.*, **32**, 47 (1926); Hess, "Die Chemie der Cellulose," p. 439.

^{40c} Hess, Weltzien, and Messmer, *Ann.*, **435**, 127 (1924); Hess, *Z. angew. Chem.*, **37**, 993 (1924).

formic,^{40d} acetic,^{40e} and oxalic^{40f} may also be used for the conversion.

Hydrocellulose, which in commercial processes plays a part similar to oxycellulose since the formation of both involves destruction of the fiber, may be distinguished from oxycellulose by certain reactions. Of these may be mentioned the liberation of carbon dioxide on distillation of oxycellulose with 12 per cent hydrochloric acid. Either hydrocellulose does not give this reaction, or the amount of carbon dioxide liberated is much smaller than that from oxycellulose.^{40g}

With concentrated acids, such as sulfuric, nitric, hydrochloric, hydrofluoric, or phosphoric, cellulose swells considerably, becomes peptized, and finally dissolves completely. Usually, immediately after preparation, the solution shows reducing power toward Fehling's and other such solutions.⁴¹ On dilution with water immediately after dissolving and with thorough cooling, the greater part of the cellulose may be regenerated in the form of white flakes which show the behavior and the x-ray diagram of mercerized cellulose (p. 1539). The preparation obtained is often also termed "amyloid," merely because it gives the same blue coloration with iodine in the presence of traces of sulfuric acid as starch (amylum) does.

Cellodextrins. On further standing of the solution just mentioned, degradation proceeds, and by addition of alcohol, a precipitate characterized by its high reducing power may be obtained. It is partly or entirely soluble in water and has, under the name of "cellulose dextrins" or "cellodextrin" played a great part in the endeavors to isolate homogeneous intermediates in the course of the degradation of cellulose. In the light of the modern conception of the chemical constitution of cellulose, cellulose dextrin is far from being a homogeneous product. It is rather to be regarded as a mixture of more or less shortened glucose anhydride chains; in other words, a mixture of oligosaccharides of varying chain length, the longest link of which may comprise thirty or less glucose anhydrides.⁴² Since the molecules of smaller size crystallize, the whole mass appears crystalline.⁴³ Certain biose anhydrides, which at one time were claimed to have been isolated as homogeneous degradation intermediates, also apparently represent mixtures of

^{40d} Heuser and Schott, *Cellulosechem.*, **6**, 10 (1925); Staudinger and Dreher, *Ber.*, **69**, 1733 (1936).

^{40e} Reference 6c, p. 171.

^{40f} Heuser and Eisenring, *Cellulosechem.*, **4**, 13, 25 (1923).

^{40g} Heuser and Stöckigt, *ibid.*, **3**, 61 (1922).

⁴¹ af Ekenstam, *Ber.*, **69**, 549, 553 (1936).

⁴² Meyer and Mark, *Ber.*, **61**, 2432 (1928).

⁴³ Freudenberg, *Ber.*, **62**, 383 (1929).

oligosaccharides of various chain lengths similar to the cellulose dextrins.^{43a}

Oligosaccharides. Isolation of the oligosaccharides is best accomplished on acetolysis or on hydrolysis preceded by methylation or on methylation after acetolysis. The products of reaction are more stable in these cases, and their separation is thus facilitated. A method of effecting this hydrolysis is that of Zemplén, using methyl alcohol and a trace of sodium methylate.^{43b} Since on acetolysis the hydroxyl groups steadily increase in number and are simultaneously acetylated, because of the progressive opening of oxygen bridges, the increase in acetyl content provides a general means of following the process of degradation.^{15b} Of the oligosaccharides, the following have been isolated: cellohexaose, cellotetraose, cellotriose, and the disaccharide, cellobiose. A mixture of them may be obtained by hydrolysis with highly concentrated hydrochloric acid (specific gravity 1.21 at 15°), but the process must be interrupted before it has gone too far. This is accomplished by adding ethyl alcohol, which is used also for fractionating the mixture.⁴⁴

Even cellohexaose, $C_{36}H_{62}O_{31}$, the highest member so far isolated, crystallizes in very fine but homogeneous needles; its molecular weight corresponds to the formula given above, and although it has not yet been possible to obtain crystalline derivatives of the hexasaccharide, some of the other oligosaccharides, cellotetraose, $C_{24}H_{42}O_{21}$, and cellotriose, $C_{18}H_{32}O_{16}$, are known. They yield well-identified acetates, permethylates,⁴⁵ and osazones; the last-named, however, are difficult to obtain in crystalline form. A cellotrioside, decamethyl- β -methyl-cellotrioside, has been synthesized from 2,3,6-trimethyl- β -methylglucoside and heptamethyl-1-chlorocellobiose.⁴⁶

From a mixture of the reaction products, cellobiose ($C_{12}H_{22}O_{11}$) (p. 1465) and the last link of the chain, *d*-glucose, may also be isolated. It is interesting to note that for a long time cellobiose was not discovered among the products of ordinary hydrolysis with mineral acids. It is more easily obtained on acetolysis in the form of its octaacetate,^{46a} $C_{12}H_{14}O_{11}(C_2H_3O)_8$; potassium or sodium cellobiosate may be obtained by saponification with alcoholic potassium or sodium hydroxide, and by the action of acetic acid cellobiose may be isolated. Besides the octaacetate of cellobiose a certain amount of pentaacetyl-*d*-glucose is

^{43a} Bergmann and Knehe, *Ann.*, **445**, 1 (1925); Hess and Friese, *Ann.*, **450**, 40 (1926).

^{43b} Zemplén, *Ber.*, **59**, 1254 (1926); Zemplén and co-workers, *Ber.*, **69**, 1827 (1936).

⁴⁴ Zechmeister and co-workers, *Ber.*, **64**, 857 (1931); **66**, 269 (1933).

⁴⁵ Freudenberg and co-workers, *Naturwissenschaften*, **18**, 1114 (1930); *Ann.*, **494**, 41 (1932).

⁴⁶ Freudenberg and Nagai, *Ann.*, **494**, 63 (1932).

^{46a} Skrap and König, *Ber.*, **34**, 1115 (1901); *Monatsh.*, **21**, 1011 (1900).

obtained.⁴⁷ Another way to arrive at cellobiose is by the action of acetyl bromide and glacial acetic acid upon cellulose at a temperature of 30–40° for a number of days. This results in acetobromocellobiose⁴⁸ which is identical with that which E. Fischer and Zemplén obtained by the action of hydrogen bromide on cellobiose octaacetate.⁴⁹

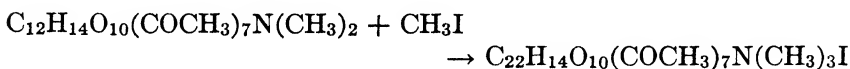
Cellobiose, for a long time the only crystalline product of cellulose degradation besides glucose, has played an important part in the attempt to clear up the chemical constitution of cellulose, as will be seen later.

Cellobiose forms an osazone; on mild oxidation it gives the monobasic cellobionic acid, which on hydrolysis yields gluconic acid and glucose. Hydrolysis of cellobiose results in *d*-glucose only. Haworth's work⁵⁰ indicates its structure as a 1-glucosido-4-glucose with a β -glucosidic linkage. This has been confirmed by a synthesis of octamethylcellobiose from tetramethylglucose-1-chlorohydrin and 2,3,6-trimethyl- β -methylglucoside.⁵¹

Cellobiose is isomeric with maltose (p. 1463) and lactose. The first two are distinguished only by different configurations which may be recognized by their behavior toward enzymes. Thus cellobiose is hydrolyzed by emulsin, the specific enzyme for methylglucosides showing the β -configuration, while maltose suffers cleavage by maltase, specific for the α -configuration.

Isocellobiose, which for a long time was considered as being formed in addition to cellobiose on hydrolysis or acetolysis of cellulose,^{51a} does not exist; it is now recognized as a mixture of cellobiose with oligosaccharides.^{51b}

Cellobiose may be converted into cellobiose anhydride by the action of trimethylamine on heptaacetyl bromocellobiose to form heptaacetylcellobiosedimethylamine, which in turn reacts with methyl iodide to form heptaacetylcellobiosidotrimethylammonium iodide.



The iodide, on treatment with dilute barium hydroxide solution, yields cellobiose anhydride. It has been obtained only once in crystalline

⁴⁷ Webber, Staud, and Gray, *J. Am. Chem. Soc.*, **52**, 1542 (1930).

⁴⁸ Karrer and Widmer, *Helv. Chim. Acta*, **4**, 700 (1921).

⁴⁹ Fischer and Zemplén, *Ber.*, **43**, 2536 (1910).

⁵⁰ Haworth, "The Constitution of Sugars," Arnold and Co., London (1929).

⁵¹ Freudenberg *et al.*, *Ber.*, **63**, 1962 (1930).

^{51a} Ost and Prosiegel, *Z. angew. Chem.*, **33**, 100 (1920); Ost and Knoth, *Cellulosechem.*, **3**, 25 (1922); Ost, *Z. angew. Chem.*, **39**, 1117 (1926).

^{51b} Freudenberg, "Tannin, Cellulose, Lignin," Springer, Berlin (1933), p. 99.

form, but there are no difficulties in crystallizing the hexaacetate. It does not reduce Fehling's solution or decolorize bromine water. Its chemical constitution has been recognized as that of 1,6,4- $[\beta$ -*d*-glucosido]-levoglucosan with the 1,6-linkage in the levoglucosan (p. 1492) constituent. The hepato-pancreas enzyme of *Helix pomatia* brings about cleavage to *d*-glucose and levoglucosan.⁵²

Cellobiose may be transformed into a great number of derivatives. A detailed discussion and description of these may be found elsewhere.^{52a}

The end product of hydrolysis of cellulose is *d*-glucose. The theoretical yield of glucose is 111.1 per cent; actually this yield has never been obtained because of further degradation of glucose to organic acids, hydroxymethylfurfural, and other substances, under the influence of the hydrolyzing means applied. The best yield may be obtained by the action of methyl alcoholic hydrochloric acid upon cellulose triacetate. Thus 95 per cent of the theoretical yield is obtained.^{52b}

The action of hydrogen bromide in ether on cellulose, in a sealed tube, leads to about 33 per cent of ω -bromomethylfurfural, a result which for a long time has given rise to speculation concerning the presence of ketonic groups in cellulose.^{52c} The primary product, however, is glucose, which under the conditions of the reaction loses water and is transformed into hydroxymethylfurfural.^{52d} In fact, the latter may be obtained directly from aldoses as easily as from ketoses.^{52e}

Anhydrous hydrogen chloride apparently converts cellulose into a mixture of polymeric glucose anhydrides (polyglucosans). On dilution with water these are transformed into glucose.⁵³ Hydrogen fluoride acts similarly, the reaction product of anhydride character having been termed "cellan." It is regarded as a product of reversion of glucose, which would mean that the latter is the primary product, possibly in the form of a labile glucosyl fluoride.⁵⁴

⁵² Karrer and Fries, *Helv. Chim. Acta*, **14**, 1317 (1931); Karrer and Harloff, *ibid.*, **16**, 962 (1933).

^{52a} See Tollens-Elsner, "Kurzes Handbuch der Kohlenhydrate," Barth, Leipzig (1935), p. 436.

^{52b} Irvine and Hirst, *J. Chem. Soc.*, **121**, 1585 (1922); Heuser and Aiyer, *Z. angew. Chem.*, **37**, 27 (1924).

^{52c} Fenton and Gostling, *J. Chem. Soc.*, **79**, 361, 807 (1901).

^{52d} Heuser and Schott, *Cellulosechem.*, **6**, 10 (1925).

^{52e} Middentrop, Doctoral dissertation, Leiden (1917).

⁵³ Schlubach and Prochownick, *Angew. Chem.*, **47**, 132 (1934).

⁵⁴ Helferich and Peters, *Ann.*, **494**, 101 (1932); Fredenhagen and Cadenbach, *Angew. Chem.*, **46**, 113 (1933).

DEGRADATION OF CELLULOSE BY THERMAL DECOMPOSITION

The products of thermal degradation, in contrast to those of hydrolysis and acetolysis, do not allow conclusions regarding the chemical constitution of cellulose. These products represent the result not only of a far-reaching degradation, but also of secondary, tertiary, and further reactions, so that it is often difficult to explain their presence.

If a temperature of about 270° is applied, as is usual in the commercial destructive distillation of wood, a great quantity of gas is produced, consisting chiefly of carbon dioxide and, particularly at higher temperatures, carbon monoxide, with smaller amounts of methane and ethylene. The process of destructive distillation of cellulose may be regarded as a carbonization with elimination of water and carbon dioxide, while carbon monoxide and all other products owe their formation to secondary, tertiary, and further reactions. The principle other products are tar and acetic acid together with some formic acid and acetone, the last two being formed long before the temperature of 270° has been reached.

Methyl alcohol, which was before its synthetic manufacture an important product of the destructive distillation of wood, owes its formation chiefly to the lignin constituent of the wood.^{54a} It is obtained from cellulose only by the process of hydrogenation in the presence of catalysts.⁵⁵

The tar produced on destructive distillation of cellulose consists chiefly of phenols, which indicates transformation of aliphatic into aromatic substances under the influence of high temperature.

Cellulose coal has not quite the same composition as anthracite, its hydrogen content being somewhat lower. However, if cellulose is subjected to heating in the presence of water, in order to avoid overheating, under a pressure of 150 atmospheres, the composition of the resulting coal approaches that of anthracite.⁵⁶

Destructive distillation of cellulose *in vacuo* yields levoglucosan or β -glucosan (yield about 38 per cent), i.e., a glucose anhydride^{56a} (p. 1488). It is identical with that which may be obtained on hydrolysis of glucosides, e.g., from picein, which splits into piceol (*p*-hydroxyacetophenone) and levoglucosan under the influence of strong bases. Its

^{54a} Büttner and Wislicenus, *J. prakt. Chem.*, **79**, 177 (1909); Heuser and Skiöldebrand, *Z. angew. Chem.*, **32**, 41 (1919); Heuser and Schmelz, *Cellulosechem.*, **1**, 49 (1920).

⁵⁵ Fierz-David and Hanning, *Helv. Chim. Acta*, **8**, 900 (1925); *Chemistry & Industry*, **44**, 942 (1925).

⁵⁶ Bergius, *Naturwissenschaften*, **16**, 1 (1928); Heuser, *Z. angew. Chem.*, **26**, 393 (1913).

^{56a} Pictet and Sarasin, *Helv. Chim. Acta*, **1**, 87 (1918); Venn, *J. Textile Inst.*, **15**, T. 414 (1924).

constitution is that of a $< 1,5 > < 1,6 > \beta$ -*d*-glucosan (see its formation on enzymatic cleavage of cellobiose anhydride). Its β -configuration is indicated by the fact that it is obtained easily from β -*d*-glucose and β -*d*-glucosides but not from α -*d*-glucose or α -*d*-glucosides.^{56b} There is no doubt that it owes its formation on destructive distillation to a secondary reaction, glucose being formed primarily. This is apparent not only from the fact that it may be obtained on destructive distillation *in vacuo* from glucose directly, but also from the fact that, on being itself subjected to this process under atmospheric pressure, the same products of distillation as from cellulose result. Levoglucosan may also be obtained from the destructive vacuum distillation of starch.⁵⁷

Levoglucosan adds one molecule of chloral to form chloral-glucose ("chloralose").^{57a} The same reaction seems to occur with cellulose itself when subjected to this treatment in the presence of pyridine. The cellulose dissolves to form a viscous liquid.^{57b}

DEGRADATION OF CELLULOSE BY MEANS OF BIOLOGICAL PROCESSES

Cellulose plays a very important part in many biological processes. Nature has provided for current destruction of cellulose by the activity of microorganisms, i.e., fungi and bacteria. By controlling the conditions of biological destruction (fermentation), valuable products such as alcohols, acids, etc., may be obtained. This forms the basis of important branches of industrial utilization of cellulose wastes.

Fermentation of cellulose eventually results in a number of gases. Depending upon the types of microorganisms used, four different kinds of fermentation, according to H. Pringsheim,⁵⁸ can be differentiated: (1) Methane fermentation, brought about by the action of sewage bacteria, produces chiefly methane, with smaller amounts of carbon dioxide and lower fatty acids (from formic to butyric). (2) Hydrogen fermentation, produced by *Bacterium fermentationis*, yields mainly hydrogen, with smaller amounts of carbon dioxide and the lower fatty acids mentioned in (1). (3) Methane-hydrogen fermentation, produced by various thermophilic bacteria, gives methane and hydrogen and smaller amounts of carbon dioxide and lower fatty acids. (4) Nitrogen fermentation, produced by denitrifying bacteria, such as are capable of

^{56b} Reference 52a, p. 515.

⁵⁷ Zemplén and Gerecs, *Ber.*, **64**, 1545 (1931).

^{57a} Pictet and Reichel, *Helv. Chim. Acta*, **6**, 621 (1923); White and Hixon, *J. Am. Chem. Soc.*, **55**, 2438 (1933).

^{57b} Ross and Payne, *ibid.*, **45**, 2363 (1923).

⁵⁸ Pringsheim, "Die Polysaccharide," Springer, Berlin (1931); Thaysen and Bunker, "The Microbiology of Cellulose, Hemicelluloses, Pectin and Gums," Oxford University Press (1927). See, however, Waksman, paper presented before the Cellulose Division of the American Chemical Society at Chapel Hill, N. C., April, 1937.

assimilating nitrogen from the air or other nitrogen sources, yields nitrogen and carbon dioxide.

As to the formation of methane, it is assumed that primarily carbon dioxide and hydrogen are liberated and these then react to form methane and water, with organic acids as intermediates.⁵⁹ However, other explanations appear possible also.

The process of fermentation is preceded by hydrolysis through hydrolytic enzymes. It has been possible to direct this process so that either cellobiose or glucose prevails by allowing either of the enzymes, cellobiase or cellulase, to act. This may be accomplished by adjusting temperature conditions to the optimum and by arresting the process of fermentation by means of antiseptics. In view of this the assumption may be made that the biological process passes through the same stages as cellulose subjected in the laboratory to hydrolysis by a chemical process. In other words, it appears likely that the bacteria also effect a gradual shortening of the cellulose chain, and that with refined methods oligosaccharides and even fractions of higher molecular weight might be isolated. Steps in this direction may be seen in the work which led to the recognition that cleavage of the longer chains (from cellulose down to the water-soluble dextrans) is apparently effected exclusively by cellulase whereas the oligosaccharides undergo scission chiefly under the action of cellobiase. Thus one may distinguish between " β -glucopolysaccharase" and " β -glucoöligosaccharase."⁶⁰

However, no connection is apparent between observations of this kind and the fact that some bacteria cease to act after they have shortened the cellulose chain to a certain extent. It would appear as though accumulation of degradation products at this stage causes the bacteria to die off.⁶¹

The number of isolated and well-defined bacteria capable of cellulose destruction has greatly increased during recent years, and very interesting results of extensive research work, especially in the field of soil microbiology, are available.⁶²

THE CHEMICAL CONSTITUTION OF CELLULOSE

Considering the facts which have just been presented in the foregoing sections it would appear that few mistakes could be made in devis-

⁵⁹ Symons and Buswell, *J. Am. Chem. Soc.*, **55**, 2028 (1933).

⁶⁰ Grassmann, Zechmeister *et al.*, *Naturwissenschaften*, **20**, 639 (1932).

⁶¹ Winogradsky, *Compt. rend.*, **183**, 691 (1926).

⁶² Waksman, "Principles of Soil Microbiology," Williams and Wilkins Co., Baltimore (1927); Waksman and Davidson, "Enzymes," Williams and Wilkins Co., Baltimore (1926); see, also, Waksman, paper presented before the Cellulose Division of the American Chemical Society at Chapel Hill, N. C., April, 1937.

ing a chemical constitution for cellulose which would be in harmony with all the facts. However, when the first attempts were made, all the facts were not known, and later attempts with more facts available, went in directions other than could *a priori* have been expected.

In order to arrive at a better understanding not only of these various attempts but also of our present conception of the chemical structure of cellulose, it appears advisable to review briefly the past history from the time the first serious suggestions were made.

At that time the following four facts were known:

1. The product of hydrolysis is glucose.
2. Acetolysis yields a tri- or disaccharide.
3. There are probably not more than three hydroxyls which react as alcoholic groups.
4. There is little indication of a free aldehyde group.

Since no other products of hydrolysis had been found, the first two facts (even though at that time cellobiose acetate, first isolated by Franchimont in 1879, was thought to be a triglucose ester) justified the definition of cellulose as a polysaccharide built up from single mono-, di-, or trisaccharides. Trained in the classical methods of approaching problems of chemical constitution, Tollens, who had established an hypothetical formula in 1895 (Fig. 1), quite naturally and logically thought of the glucose anhydrides as being linked together in accordance with the principle of polymerization in its original meaning, that is by condensation of glucose units with loss of water. It was logical and natural also that he arranged the units in a chain. The amorphous nature of cellulose, its insolubility, and other properties placed it in the class of substances of high molecular weight.

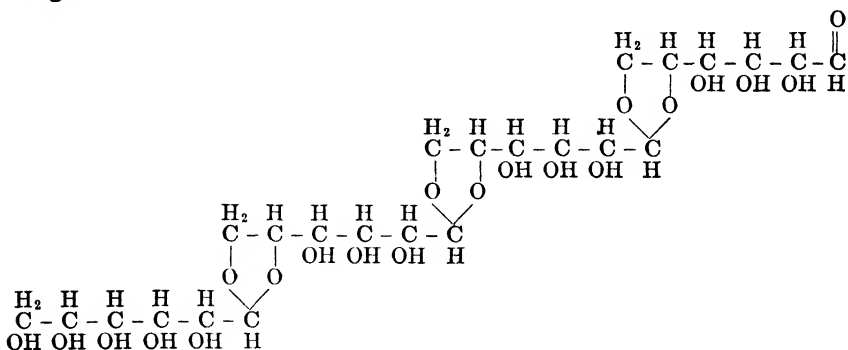


FIG. 1.—Cellulose formula. (Tollens)*

* From Heuser, "Lehrbuch der Cellulosechemie," 3rd ed., Borntraeger, Berlin (1927).

Tollens also had a very definite concept of the way in which the single units are linked together, that is the carbonyl group of one glucose unit being connected with the hydroxyls in the 5- and 6-positions of the next in the so-called double oxygen linking by which he explains the particular physical properties of cellulose. Thus, it appeared as if the only thing for the following generation to do was to deliver experimental proof for Tollens' formula and not to bother too much about the high-molecular nature. This postulation may for the moment be accepted merely as a means of showing how the development probably would have proceeded if it had not been interrupted by numerous excursions into the field of speculation concerning the nature of high-molecular substances.

Assuming that hydrolysis of cellulose yielded as the end product glucose in 100 per cent yields, the problem was to direct hydrolysis in such a way as to isolate oligosaccharides whose possible formation Tollens' formula suggested, especially since the next higher link to glucose, cellobiose, had actually been found some years before Tollens' formula was advanced, and, moreover, since it was known that nature produces tri- and tetrasaccharides, some of which had even been synthesized at that time.* Had the cellulose chemists succeeded in identifying the cellodextrins as mixtures of cellotriose, cellotetraose, pentaose, and hexaose, with still higher polymers, the road would have been opened to answer the next question, namely, how the glucose units are linked together in these oligosaccharides. In answering this question the cellobiose then actually isolated would have offered excellent material for investigation, especially since the mode of linkage between glucose molecules in other disaccharides was known to be of a glucosidic nature. The third question then would have been: Are the linkings in cellulose itself always the same, or do different linkings occur alternately?

Thus quite a number of problems were open to investigation before the final question, namely: How many glucose residues unite to form the cellulose molecule, and how many cellulose molecules unite to form the next higher aggregate and the fiber itself?

Such probably would have been the course which structural chemistry would have taken if the Tollens' generation had continued to interest themselves in this problem. However, for about twenty years very little happened except that a number of other formulas were proposed, for which, however, no new facts were available. The only result important constitutionally was the firmer establishment of the postulation

* See the enzymatic syntheses of maltose and isomaltose from glucose by Hill, *J. Chem. Soc.*, **73**, 634 (1898); **83**, 578 (1902).

that any cellulose formula must have only three esterifiable hydroxyls. This was the outcome particularly of Ost's⁶³ indefatigable studies on the acetylation of cellulose. In reality the new formulas, as for example those suggested by Vignon^{63a} and later by Green,^{63b} are only the result of a more or less well-founded speculation on the possible arrangement of the atoms in the glucose anhydrides. Besides, they do not explain the formation of cellobiose.

After the year 1920 the endeavors to establish the constitution of cellulose moved chiefly in two directions. In one it followed the solid and secure paths which for a long time had been established for the investigation of problems pertaining to the chemical constitution of molecules and which had so successfully clarified the chemical structure of many natural products. In the other direction, research left the solid ground of approved principles and entered the realm of speculation on the size of the cellulose molecule, trying to explain its nature as that of a high-molecular substance by means of a concept entirely new in the field of polysaccharide chemistry.

Röntgenography, although at its start encouraging such speculation, yet, in the main, tried to bring its findings into agreement with the constitution as determined by chemical means, and to purge itself of premature and far too vague conclusions. Thus x-ray investigation, although limited in what it can tell about the chemical side, has given much support to present knowledge of the chemical structure of cellulose and other natural products.

Before research started to follow these two directions and when x-ray investigation had just produced its first findings on the crystalline structure of high-molecular substances, a new formula was offered to the cellulose chemists in 1920 by K. Hess.⁶⁴ His formula, a pentagluco-sido-glucose, based on the ideas prevailing then concerning the structure of tannins, represented cellulose as a hexasaccharide in which five glucose units were linked, by means of glucosidic bonds, with the five hydroxyls of another glucose unit. Although this formula was entirely of a speculative nature it maintains its importance in cellulose history in having awakened a number of investigators to activity and having thus ended the long and rather fruitless period after 1895 when Tollens' formula was suggested.

Hess's formula would account for a yield of only 32-33 per cent cellobiose and thus was not in agreement with the 40 per cent yield

⁶³ See Hess, "Die Chemie der Cellulose," or Heuser, "Lehrbuch der Cellulosechemie," 3rd ed., Borntraeger, Berlin (1927).

^{63a} Vignon, *Bull. soc. chim.*, [3] **21**, 599 (1899).

^{63b} Green and Perkin, *J. Chem. Soc.*, **89**, 811 (1906).

⁶⁴ Hess, *Z. Elektrochem.*, **26**, 232 (1920).

actually obtained. This stimulated Karrer^{64a} and Freudenberg, independently of each other, to study anew the acetolysis of cellulose. Freudenberg⁶⁵ calculated that not 40 but 61 per cent of cellobiose is formed and that this 61 per cent could be isolated if it were not for the fact that about 20 per cent is lost because of hydrolysis to glucose under the conditions of acetylation. A calculation based on a chain length of 100 units revealed that probably the highest amount which can exist in the mixture after acetolysis is 67 per cent. Thus, while the pentaglicosido-glucose formula was not in agreement with one of the most important facts, the results of acetolysis pointed in the direction of a long chain, i.e., the principle put forward in Tollens' suggestion.

At about the same time, the method of total methylation and hydrolysis of the methylated products, as applied by Irvine, by Haworth, and by Karrer and their collaborators (p. 1553), proved an excellent means of obtaining information on structural problems.

The interesting results which were thus produced on the probable structures of maltose, cellobiose, and other disaccharides (as well as the results obtained by Karrer by the use of phosphorus pentabromide) were over-shadowed by the endeavors to find another explanation for the high-molecular state of organic natural products. These endeavors can be traced back to Cross and Bevan,⁶⁶ pioneers in the cellulose field, who expressed the opinion that cellulose may be an aggregation of units different from ordinary molecules. The issue was raised anew by Hess's formula, which brought to the fore those investigators who did not see any necessity for changing the classical concept of chemical constitution in the case of highly polymeric substances.

With his formula Hess at the same time had offered the suggestion that the cellulose molecule is represented by a number of hexasaccharide units held together by means of "residual forces of affinity." This idea, likely influenced by Werner's coördination theory of the nature of complex molecules, possibly because it was a deviation from the previously accepted meaning of polymerization, appeared to many to offer a welcome possibility for widening their knowledge in this respect. From 1920 on the idea of the small units held together by forces other than normal valences gained much headway.

With the pentaglicosido-glucose formula abandoned, it was assumed that the anhydrides of glucose or perhaps cellobiose, were possible "building units" of cellulose. Thus, Karrer,⁶⁷ in 1921, expressed the

^{64a} Karrer and Widmer, *Helv. Chim. Acta*, **4**, 174 (1921).

⁶⁵ Freudenberg, *Ber.*, **54**, 767 (1921).

⁶⁶ Cross, Bevan, and Traquair, *Chem. Ztg.*, **29**, 527 (1905).

⁶⁷ Karrer, "Polymere Kohlenhydrate," Akad. Verlags-Ges., Leipzig (1925); Karrer, *Cellulosechem.*, **2**, 127 (1921).

strong belief that the anhydroglucoses in starch as well as those in cellulose are held together by means of "secondary bonds" and that depolymerization does not involve such structural changes as the opening of oxygen linkings.

For Karrer the building unit of cellulose was an anhydrocellobiose, and the "exponent of polymerization" (degree of polymerization), deduced from the heat of combustion of starch, and from a general analogy with starch, was thought to be not greater than three and probably two. Consequently Karrer considered the cellulose molecule to be a dimeric cellobiose anhydride $(C_{12}H_{20}O_{10})_2$. This formula denies the possibility that hydrolysis may produce other oligosaccharides than one, namely cellobiose, a postulation which was in agreement with Karrer's strict refusal to believe a chain structure possible.⁶⁷

That the cellulose molecule should be represented by only two cellobiose anhydrides held together by secondary valences, instead of a hundred or more of such units linked together by means of normal valences, certainly was a daring suggestion, even in the memorable year 1921 when the first results of x-ray investigation were thought to be not in disagreement with the conception of the small units. R. O. Herzog and Jancke⁶⁸ had found that the group $(C_6H_{10}O_5)_4$ repeated itself regularly within the space lattice pattern, obtained from monochromatic x-radiation of cellulose fiber, a result which was welcomed by Karrer as being in good agreement with his conception. This shows that at that time x-ray possibilities were much overestimated; the elementary unit, that is, the basic cell of the crystal, was confounded with the molecule.

Hess went still further than Karrer when he claimed that the cellulose molecule is monomeric, i.e., it is represented by a single glucose anhydride. It was assumed by Hess to exist as such in solution,⁶⁹ for instance in cuprammonium solution, and as was claimed a few years later (1926 and the following years), also in the form of derivatives, such as acetyl- and methylcellulose in their respective solutions. In its solid form, and also in that brought about by regeneration from solution, cellulose then would represent an association of monomeric glucose anhydrides, and this association would be held together by secondary valences. The idea of association was thus substituted for that of polymerization.

In Karrer's cellulose structure representing a dimeric anhydrocellobiose, the two anhydrocellobiose units were also thought to be held together by secondary valences, and a great number of the dimeric molecules were thought to be united, by means of "crystal" valences, to build up the cellulose itself. Depolymerization then would first force the

⁶⁸ Herzog, *Z. physik. Chem.*, **139**, 235 (1928).

⁶⁹ Hess, "Die Chemie der Cellulose," pp. 294, 400, 432, and 448.

crystal valences to open and single cellulose molecules would result. The next step would be the breaking of the secondary bonds, to yield single anhydrocellobiose molecules. On hydrolysis these would result in cellobiose and on further hydrolysis, in glucose. Although the opinion was expressed that with cellulose depolymerization can scarcely occur without hydrolysis at the same time, Hess⁷⁰ claimed to have obtained (by means of acetyl chloride) an anhydro biose as a monomeric individual, a claim which seemed to receive much support by the isolation, reported by Bergmann and Knehe,⁷¹ of another anhydro biose.

Without accepting the idea of small units, the concept of depolymerization was further developed⁷² when attempts were made to explain the fate of cellulose during a number of technical processes, such as the conversion of cellulose into solutions and the regeneration to artificial silk, films, and the like, or the isolation of cellulose from plant material by the various processes of pulping, bleaching, etc. It was assumed that in all these processes larger complexes are broken up into smaller ones without change in the chemical structure of the molecule. The degree of depolymerization, which was also termed "physical depolymerization," depends upon the intensity of the means applied, and expresses itself in changes in various properties.

It appeared plausible to assume that in the various processes, supermolecular forces, which hold the molecules together in the various forms in which cellulose appears (e.g., fiber and amorphous cellulose), are loosened and broken down, resulting in smaller complexes, and that this happens long before a change in the chemical structure of the cellulose can be detected.

In the meantime, methylation of cellulose, which Denham had begun in 1913, was made the subject of renewed studies. Hydrolysis yielded trimethylglucose, whose structure as a 2,3,6-methylated monose was recognized by Denham, confirmed by Haworth and Leitch, and later by Irvine and Hirst.^{72a} This recognition, together with the fact that Irvine and Hirst could obtain a yield of about 80 per cent of 2,3,6-trimethylglucose, was important since it showed that in cellulose, the hydroxyls in the 1- and 4-positions of each glucose unit must be occupied. With cellobiose known as one of the intermediates of hydrolysis, there was no doubt that one hydroxyl, probably that in the 4-position of one glucose unit, was glucosidically linked with the other glucose unit of the cellobiose. It was only natural to assume that in this second unit

⁷⁰ Hess and Friese, *Ann.*, **450**, 40 (1926).

⁷¹ Bergmann and Knehe, *Ann.*, **445**, 1 (1925).

⁷² Heuser, "Lehrbuch der Cellulosechemie," 3rd ed. (1927), p. 264.

^{72a} Irvine and Hirst, *J. Chem. Soc.*, **121**, 1585 (1922); **123**, 518 (1923).

(the non-reducing component) the hydroxyl in the 4-position was connected in the same type of linkage with a third glucose unit (see Figs. 2, 3, and 4). Hence it was concluded that cellulose must consist of at least three glucose anhydrides. On this assumption acetolysis should theoretically yield two-thirds cellobiose and one-third glucose. But whether the linking was a 1,4- or a 1,5- could not yet be told with

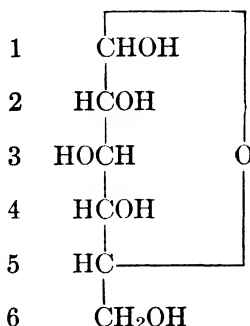


FIG. 2.—Glucose (Amylene oxide form)

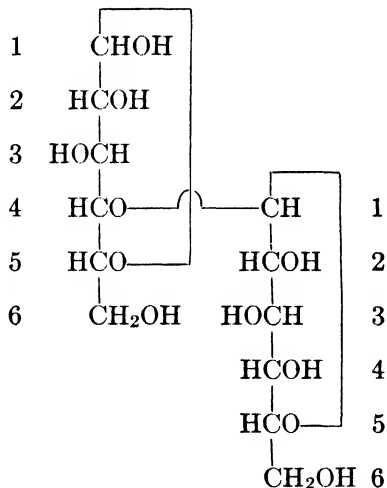


FIG. 3.—Cellobiose (1,4-Linking)

certainty. It may be mentioned here that in Tollens' chain formula the hydroxyls in the 2-, 3-, and 6-positions are available for methylation (or acetylation, etc.) while the 1-, 4-, and 5-positions are involved in the linking of the individual glucose units (Fig. 5). Consequently Tollens' old and much-contested formula could well explain the formation of 2,3,6-trimethylglucose from trimethylcellulose on hydrolysis.

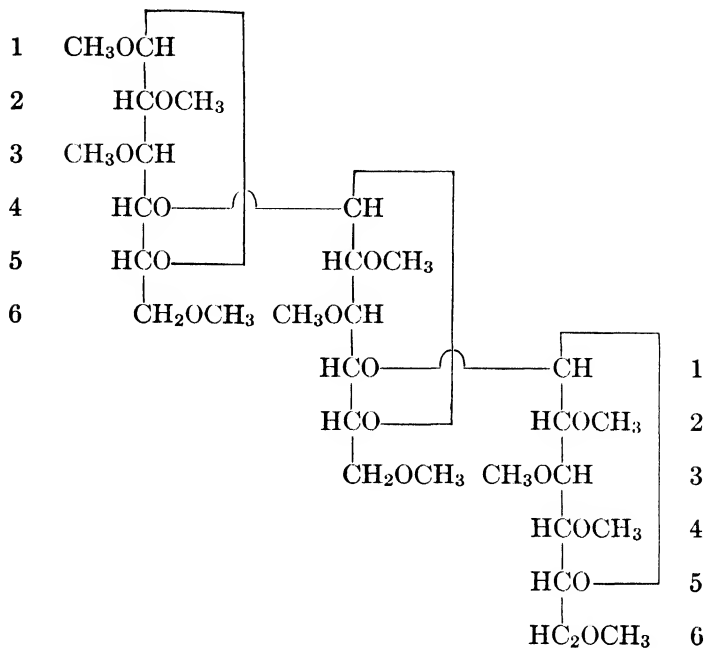


FIG. 4.—Methylated cellotriose

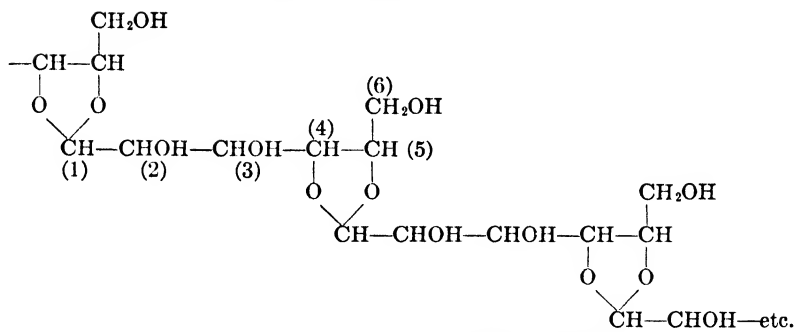


FIG. 5.—Cellulose formula (Tollens)

The influence of the idea of the small molecules reached even the rather conservative English chemists. Irvine and Hirst suggested that the cellulose molecule was built up of three glucose anhydrides of butylene oxide structure connected to each other by 1,5-linkages to form a cyclic trisaccharide.

Ring structure was assumed because of the failure to detect any tetramethylglucose in the degraded methylation products. An open chain naturally must have a beginning and an end, and the terminal units can each be linked to only one neighboring glucose. This being

the case, it follows that in each of the two terminal units there should be one more reactive group open to methylation (see Fig. 4). In the reducing unit it is the reducing group which is open to methylation, and in the non-reducing unit, it is the hydroxyl in the 4-position which may be methylated. On hydrolysis the methoxyl of the reducing group is eliminated and the reducing group is regenerated, but the methoxyl group in the other terminal unit remains. Thus, hydrolysis would have to yield a certain percentage of tetramethylglucose. Since this could not be found, ring structure was assumed.

Here may be mentioned Hibbert's⁷³ suggestion made about one year earlier. His formulation of the glucose anhydride was the first attempt to account for the exclusive formation of 2,3,6-trimethylglucose (on hydrolysis of methylcellulose), and it is interesting that his formulation is identical with that of the β -glucosan which is formed on distillation of glucose *in vacuo*. Hibbert left the number of glucose anhydride units open for discussion.

It must be emphasized that Denham, when he made his second investigation as early as 1917, had found a trace of a crystalline substance which "resembled tetramethylglucose." Although he did not pay much attention to it he thought it possible that tetramethylglucose might be "a normal product of hydrolysis of methylated cellulose and that cellulose itself may chiefly be represented by an open chain of condensed glucose radicals."

On the other hand, the search for the tetramethylglucose was never given up. As already mentioned, Irvine, together with Hirst,^{73a} looked for it in vain and so did others after them (see for instance Freudenberg *et al.*⁷⁴), and it was not until 1932 that Haworth and Machemer⁷⁵ isolated it. The fact that tetramethylglucose had not been found, although its presence had been dimly indicated, was used as one of the arguments against the chain structure, particularly in Germany where the idea of the small units had been born and was being fostered more than anywhere else.

In general, the English chemists did not bother much about the new conception, especially since Sponsler⁷⁶ and other x-ray investigators had made it clear that the basic cell of the crystal lattice and the molecule of cellulose are two different things, and were steadily recognizing the

⁷³ Hibbert, *Ind. Eng. Chem.*, **13**, 256 (1921); *J. Chem. Soc.*, **119**, 803 (1921).

^{73a} Irvine and Hirst, *ibid.*, **123**, 529 (1923).

⁷⁴ Freudenberg and co-workers, *Ann.*, **460**, 288 (1928); **494**, 54 (1932).

⁷⁵ Haworth and Machemer, *J. Chem. Soc.*, 2372 (1932); *Trans. Faraday Soc.*, **29**, 14 (1933); *Nature*, **129**, 365 (1932).

⁷⁶ Sponsler, *J. Gen. Physiol.*, **9**, 221 (1925); **10**, 677 (1926); see, also, *Phys. Rev.*, [2] **10**, 661 (1917).

necessity of bringing their evidence into harmony with the structure deduced from chemical experiments which pointed strongly to a linear molecule.

At this time the constitutions of cellobiose and isomeric disaccharides such as maltose, etc., were the focus of interest. In 1926, Haworth, Charleton, and Peat^{76a} gave proof of the constitution of cellobiose as a 1-glucosido-4-glucose (Fig. 3), and in the same year Zemplén arrived at the same structure by way of a cyanohydrin synthesis.^{76b} As Haworth had demonstrated *d*-glucose to have a pyranose formula, cellobiose could be formulated as in Fig. 6. The importance of the clarification

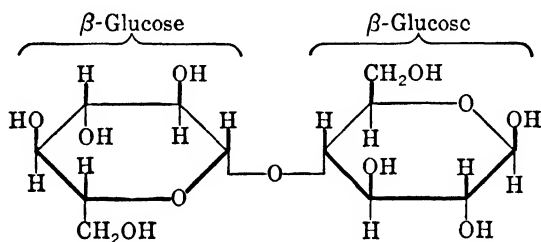


FIG. 6*

of the cellobiose structure may be expressed in Haworth's words: "The elucidation of the ring structure of sugars gave a new impetus to constitutional study, and the allocation of the hexagon formula to glucose provided new interpretations of the experimental evidence bearing on the constitution of the polysaccharides."⁷⁷

It was a fortunate coincidence that at about the same time (1926) the search for oligosaccharides higher than cellobiose among the products of cellulose hydrolysis had been successful. As early as 1923 Bertrand and Benoist^{77a} had described a triose among the products of acetolysis which was confirmed by Ost^{77b} and by Irvine and Robertson.^{77c} Today it is known that the triose and the tetraose found among the products resulting from hydrolysis with strong hydrochloric acid had been isolated by Willstätter and Zechmeister in 1913.⁷⁸ Irvine and Robertson obtained the trisaccharide by interrupting the

^{76a} Charlton, Haworth, and Peat, *J. Chem. Soc.*, **129**, 89 (1926).

^{76b} Zemplén, *Ber.*, **59**, 1254 (1926).

* From Haworth, "The Constitution of Sugars," Arnold and Co., London (1929). (Courtesy of the publishers.)

⁷⁷ Haworth, "The Constitution of Sugars," p. 74.

^{77a} Bertrand and Benoist, *Compt. rend.*, **177**, 85 (1923); **176**, 1583 (1923).

^{77b} Ost, *Z. angew. Chem.*, **39**, 1117 (1926).

^{77c} Irvine and Robertson, *J. Chem. Soc.*, **123**, 1488 (1926).

⁷⁸ Willstätter and Zechmeister, *Ber.*, **62**, 722 (1929).

acetolysis immediately before the formation of the octaacetate of cellobiose occurs, that is, before the last step in the formation of cello-dextrins is reached.

Probably this is a somewhat later stage than that from which Hess, and Bergmann and Knehe believed they had isolated a biose anhydride (p. 1573) which later proved to be a mixture.

The formation of anhydrides when cellulose is exposed to certain reactions, is of course quite possible, but these anhydrides no doubt owe their formation to secondary reactions. As in the case of levoglucosan, it must be assumed that hydrolysis to mono- or disaccharides is the primary reaction and that the loss of water follows.

These, also, are the phases of the reaction that takes place on treatment of trimethylcellulose with hydrogen chloride in ether which, according to Freudenberg and Braun,⁷⁹ yields 1-chloro-2,3,6-trimethylglucose. On removal of the chlorine by means of metallic sodium, the intermediate is converted into 2,3,6-trimethylglucose anhydride (Fig. 7).

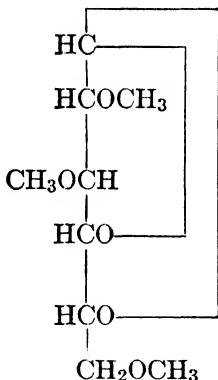


FIG. 7

2,3,6-Trimethylglucose anhydride

This anhydride behaves very differently from trimethylcellulose; it has a melting point, a molecular weight in accordance with its monomeric formula, and it lacks any ability to associate to form higher aggregates. Hence, there is no reason to ascribe to the glucose anhydride and other *lower* anhydrides obtained from cellulose supermolecular forces that would effect association of single anhydrides to cellulose itself, as postulated in Hess's hypothesis of the structure of cellulose.

On the other hand, some observations pointed in the direction of polymerization of anhydro sugars to complex compounds. For instance,

⁷⁹ Freudenberg and Braun, *Ann.*, **460**, 288 (1928).

Pictet and Ross^{79a} observed such a polymerization with β -glucosan upon heating it to a high temperature in the presence of zinc chloride. Another example is the reassociation of the triacetate of a glucose anhydride in aqueous solution to a cellulose-like substance, called "cellosan," reported by Pringsheim.^{79b} Such observations no doubt suggested the possibility of molecular aggregation by forces other than those which are utilized for synthesizing di-, tri-, and higher saccharides. But in respect to Pictet's β -glucosan no clear relationship of the complex bodies to cellulose has been established, and in the association of Pringsheim's glucose anhydride to cellosan it is doubtful whether the primary process, namely the degradation of the cellulose into a glucose anhydride on heating in naphthalene, had actually gone that far, since the determination of the molecular weight in the usual way, on which the conclusion was based, is not reliable.

In the light of the conception of physical depolymerization, as indicated before, the hypothesis was further developed that such processes first break the bonds which hold the chain units together laterally to form their higher aggregates, the micellae. If the processes are sufficiently severe, depolymerization will not cease at these bonds but will proceed further and attack the chains, splitting them into shorter molecules. The products of such cleavages, however, are still so large that the characteristics of high-molecular substances are still evident.

An attempt to illustrate this principle was made using a series of increasingly degraded cellulose preparations, from native cotton cellulose, regenerated cellulose, hydrocellulose, down to the cellodextrins, and it was shown that the degree of depolymerization clearly depends upon the previous treatment to which the cellulose has been subjected.⁸⁰ This principle of depolymerization was demonstrated also by Staudinger, at about the same time,⁸¹ on cellulose acetates of varying degree of depolymerization.

The processes of depolymerization may sometimes occur when the original intact cellulose is forced to go into solution. On regeneration, the fiber structure is lost, and "amorphous" cellulose is obtained with physical properties very different from those of the original substance. But the changes in chemical structure which may have taken place on depolymerization, either before or during the process of dis-

^{79a} Pictet and Ross, *Compt. rend.*, **174**, 1113 (1922); Pictet and Sa'zmann, *Helv. Chim. Acta*, **8**, 948 (1925); Irvine and Oldham, *J. Chem. Soc.*, **127**, 2903 (1925).

^{79b} Pringsheim and co-workers, *Ber.*, **58**, 2135 (1925).

⁸⁰ Heuser, *Z. Elektrochem.*, **9**, 498 (1925); Heuser and Hiemer, *Cellulosechem.*, **6**, 101, 125, 154 (1925). See, also, Heuser, "Rapports sur les hydrates de carbone," 10e conférence de l'union internationale de chimie, Liège (1930); Paris (1930), pp. 207 *et seq.*

⁸¹ Staudinger, "Die hochmolekularen organ. Verbindungen," Springer, Berlin (1932).

solving, have not yet become measurable, for the shortened chains are still so long that the usual means of chemical identification fail. As for the process of association, i.e., the spontaneous agglomeration which is frequently observed in solutions of cellulose derivatives which have stood for a certain length of time, it may well be permitted to endow the shortened anhydride chains in solution with supermolecular forces which, under certain circumstances, may cause the reassociation of single chains of various lengths into "bundles."

But these forces must not be ascribed to the lower anhydrides which can be identified chemically. For quite a large number of these must be linked together (and it is important to note that this linking occurs through primary valences) before the physical properties of the thus enlarged molecule become such as are characteristic of high-molecular substances. These are the colloidal nature, the failure to crystallize, the high viscosity in solution, the ability to form films, and the ability to reassociate. It will be remembered that a classical example was supplied by Emil Fischer in the synthesis of increasingly larger polypeptides. But not until a molecular weight of about 1200 was reached did the polypeptide behave like a colloidal substance.^{81a} Later, Staudinger⁸¹ illustrated this principle in his synthetic polymeric series of long-chained molecules, such as the polyhydroxymethylenes (and -ethylenes), the polystyrenes, and the polyvinyl acetates. These polymeric series, after having reached a certain chain length, are quite comparable with cellulose as far as their physical properties and colloidal behavior are concerned.

The comparability of the cellulose molecule and other naturally occurring polymeric substances with synthetic polymers was also most interestingly demonstrated by a study of polyamides and polyacetates, which were synthesized by Carothers⁸² and his collaborators. With artificial threads obtained from such polymers, Carothers was able to demonstrate that a useful degree of strength and pliability is reached only with a minimum molecular weight of about 12,000 and a chain length of not less than approximately 1000 Å (1 Ångström unit = 10^{-8} cm. = 0.1μ).

From the foregoing it is evident that supermolecular forces become active only when the molecule has attained a certain size. In other words, the development of such forces, as mysterious as this phenomenon may seem, is a consequence of the growing molecule. The primary valences do not suffice to keep so many units tightly enough together,

^{81a} Fischer, *Sitzber. preuss. Akad. Wiss. Physik. math. Klasse*, 990 (1916).

⁸² Carothers and associates, *J. Am. Chem. Soc.*, **51**, 2548, 2560 (1929); **52**, 314, 711, 3292 (1930); **54**, 1559, 1566, 1569, 1579 (1932).

and assistance is needed. This is supplied by the supermolecular forces.

The fact that these forces are a function of the growth of the molecule may be regarded as strong support for the belief that the dispersion of cellulose in solution does not separate the chains into monomolecular anhydrides, nor does it seem justified to assume that these forces are of a latent character in the lower anhydrides.

Most probably, the plant, the great master, builds up the high-molecular saccharides, such as cellulose, starch, the pentosans, and inulin, according to the same principle which prevails during synthesis in the laboratory; that is to say, it uses the primary valences of the simple compounds for synthesizing the disaccharides and the oligosaccharides, stabilizes them by dehydration and thereafter leaves it to the supermolecular forces to endow the longer and longer growing chains with still greater stability.

It appears very plausible to identify these forces with the "van der Waals' forces of molecular cohesion," a conception to which van Laar⁸³ apparently was the first to direct attention. These forces are assumed to increase with the size of the molecule. Meyer and Mark⁸⁴ attempted to estimate these forces for various chain lengths.

With regard to the identity of cellulose in plants there seems to be no doubt that different plants (and perhaps even the same plant) are capable of polymerizing single units into chains of *different* lengths, and it appears quite certain that this view would be confirmed by differences in viscosity in solution or by other means, if the chains of various lengths could be isolated without degradation.

In this connection attention may be directed to the biological polymerization which results from the action of certain bacteria on sugars. Cellulose produced from sugars by *Acetobacter xylinum*, as recently shown by Hibbert⁸⁵ is chemically the same as plant cellulose. It also gives, as G. L. Clark,⁸⁶ and later, Champetier⁸⁷ and Khouvine⁸⁸ have shown, the same x-ray diagram as plant cellulose. It, therefore, should be excellent material for elucidating the question of how many glucose anhydrides these bacteria are able to unite; in other words, whether

⁸³ See Freudenberg, "Tannin, Cellulose, Lignin," Springer, Berlin (1933), p. 98 and p. 105, footnote 2.

⁸⁴ Meyer and Mark, *Ber.*, **61**, 593 (1928).

⁸⁵ Hibbert and Barsha, *Can. J. Research*, **5**, 580 (1931).

⁸⁶ Clark, "Applied X-Rays," 2nd ed., McGraw-Hill, New York (1932), p. 445.

⁸⁷ Champetier, *Ann. chim.*, [10] **20**, 5 (1933).

⁸⁸ Khouvine, "Actualités scientifiques et industrielles," No. 164, II; Khouvine, "Cellulose et bacteries," Herman & Co., Paris (1934); *Compt. rend.*, **196**, 1144 (1933); **198**, 1544 (1934).

their ability is limited compared with that of plants. It might be possible to arrest the activity of the bacteria by some means so that they would build up chains of limited length. This and the isolation of such chains of increasing length would be a valuable means of throwing light upon the synthesis of cellulose from its building units. The technique involved here would probably offer fewer difficulties than an attempt to interrupt the synthesis of cellulose as it occurs in plants.

With the concepts of polymerization and depolymerization explained, and with the chemical evidence leaving scarcely any doubt that cellulose consists of a number of glucose anhydrides arranged in a chain, the next question was whether the linkings between the individual units alternate or are all the same.

It will be remembered that derivatives of cellobiose as well as of cellotriose could be obtained synthetically by allowing 2,3,6-trimethyl- β -methylglucoside to react on the chlorohydrins of permethylated glucose (tetramethylglucose-1-chlorohydrin) and cellobiose (heptamethylcellobiose chlorohydrin).⁸⁹ By these syntheses from components in which the types of linkage were known, and by determination of the optical rotation values and by quantitative evaluation of the optical superposition, Freudenberg was able to prove that the two glucosidic linkages in the cellobiose, the three in the cellotriose, the four in the cellotetraose, and the six in the cellohexaose, all belong to the β -series. Hence, it is very probable that cellulose too contains only β -linkages. One α -linkage, like that in maltose, to one hundred β -linkages might reveal itself by a perceptible change in the molecular rotation.

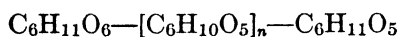
Further proof for the fact that there is only one type of linkage between the individual glucose units of the cellulose molecule was brought forth by a study of the kinetics of the cleavage as effectuated by hydrolysis and acetolysis. As these reactions proceed with time, more and more carbonyl groups are exposed, the number of which may be quantitatively estimated. Thus, the extent to which cleavage occurs may be expressed as a function of time. Since the di- and oligosaccharides submit to hydrolysis more easily than the longer chains, the premise of the kinetic calculation, namely, that all linkages undergo cleavage with the same ease, is not quite fulfilled. Yet, a decision could be made as to whether the linkages are all the same or whether different linkages alternate.

After the fundamentals of such investigations had been established

⁸⁹ Freudenberg and Nagai, *Ann.*, **494**, 63 (1932); Freudenberg and co-workers, *Ber.*, **63**, 1961 (1930).

by Meyer, Hopff and Mark,⁹⁰ and by Kuhn,⁹¹ Freudenberg's⁹² numerous investigations during recent years leave no doubt that the linkages in cellulose are all of the same type.

Thus, at the present time cellulose is regarded as a chain in which a large number of glucose units are linked together in a mode corresponding to that which occurs in cellobiose, namely, through the one and four positions. It must be assumed that the chain is open, which means that there is no linking between the two terminal units, the first unit having its hydroxyl in the 4-position and the end unit its hydroxyl in the 1-position free. This may be expressed in writing the condensed cellulose formula thus:



Finally, the length of the chain, that is, the total number of glucose units, remains to be discussed.

In a previous section (p. 1576) it has been mentioned that the permethylated cellulose yields on hydrolysis, according to Haworth and Machemer,⁷⁵ trimethylglucose and a certain amount of tetramethylglucose (0.6 per cent), indicating that the non-reducing glucose unit at the end of the chain possesses four hydroxyl groups open to methylation. The additional hydroxyl is that in the 4-position of the unit at the left-hand side of the formula (Fig. 8). The proportion of tetra-

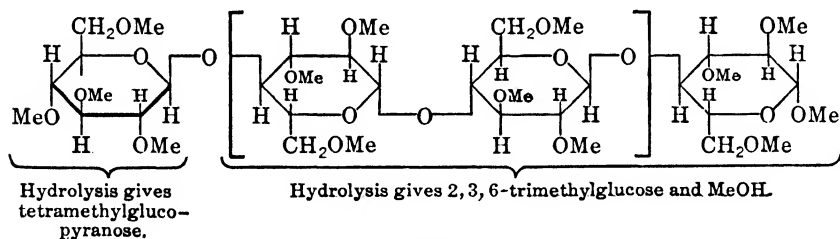


FIG. 8*

methyl- to trimethylglucose thus furnishes an approximate measure of the length of the chain. Haworth and Machemer have calculated it to consist of not fewer than 100 and not more than 200 glucose units, and they regard this size to be the average lower limit of the cellulose mole-

⁹⁰ Meyer, Hopff, and Mark, *Ber.*, **62**, 1103 (1929); **63**, 1531 (1930).

⁹¹ Kuhn, *Ber.*, **63**, 1503 (1930).

⁹² Freudenberg *et al.*, *Ber.*, **63**, 1510 (1930); Freudenberg, "Tannin, Cellulose, Lignin," pp. 99 *et seq.*; Freudenberg and Blomqvist, *Ber.*, **68**, 2070 (1935); *Trans. Faraday Soc.*, **32**, 75 (1936); Blomqvist, *Sitzber. heidelberg. Akad. Wiss. math. naturw. Klasse*, **7** (1936); Freudenberg, *Monatsh.*, **69**, 144 (1936).

* From Haworth, "The Constitution of Sugars," Arnold and Co., London (1929). (Courtesy of the publishers.)

cule.^{92a} The corresponding molecular weight of the cellulose would be between 20,000 and 40,000.

This method of determining the length of the chain is of course dependent upon the past history of the cellulose preparation used. It gives an idea of the latter's chain length with the reservation that the method of hydrolysis as well as that of isolation of the tetramethyl product are only approximately quantitative.⁹³

The other methods in which attempts are made to calculate the chain length based upon the special significance of a terminal unit or terminal groups must be viewed with a similar reservation. This is true of Bergmann and Machemer's⁹⁴ method based upon determination of the carbonyl group in the terminal unit and also of E. Schmidt's method,⁹⁵ which assumes certain free carboxyl groups in the cellulose molecule. Schmidt, by conductometric titrations, finds the amount of carbon dioxide liberated (0.28 per cent) to be a constant for most of the cotton samples investigated. If carbon dioxide actually originates from the cellulose and not from impurities it would be necessary to assume the terminating groups of cellulose to be carboxyls, and cellulose would have to be regarded as an acid. Using the value of 0.28 per cent of carbon dioxide as a basis, Schmidt calculated native cellulose (raw cotton freed of wax and fat by extraction) to consist of 96 C₆-units. However, raw cotton, even extracted, cannot be regarded as chemically pure cellulose; it contains for example, a certain amount of pectin, a substance in which galacturonic acid plays an important part, and possibly other non-cellulosic substances. These may be the cause of the liberation of carbonic acid.

Of the physical methods of determining molecular weight it must be said that the classical methods which serve so well for ordinary organic compounds fail for high-molecular substances. More recently a number of other physicochemical methods have been developed. Thus the osmotic-pressure method (measured on cellulose derivatives) indicates molecular weights which range between 25,000 and 100,000 (between

^{92a} Haworth, *Monatsh.*, **69**, 314 (1936).

⁹³ Hess, *Angew. Chem.*, **49**, 841 (1936), was not able to obtain tetramethylglucose from carefully purified cotton cellulose and believes that the small amounts isolated by Haworth are due to the fact that Haworth used acetylated cellulose. Thus, it is said, the presence of tetramethylglucose may be traced to an acetolytic action on the cellulose prior to methylation. See, also, Hess and Neumann, *Ber.*, **70**, 710, 721, 728, 734 (1937).

⁹⁴ Bergmann and Machemer, *Ber.*, **63**, 316, 2304 (1930); Staudinger and Schweitzer, *Ber.*, **63**, 3132 (1930); Staudinger, "Die hochmolekularen organischen Verbindungen"; Ulmann, "Molekülgrößen-Bestimmungen hochpolymerer Naturstoffe," Steinkopff, Dresden (1936), p. 100.

⁹⁵ Schmidt *et al.*, *Ber.*, **69**, 366 (1936); see, also, Lüttke, *Biochem. Z.*, **268**, 372 (1934).

155 and 600 glucose units), depending upon the degree of depolymerization through which the various preparations have passed. Besides, evaluation of the results has revealed lack of homogeneity in most of the derivatives.^{95a}

According to Staudinger,⁹⁶ the relation between solution viscosity of cellulose in cuprammonium solution and its molecular weight (chain length) is of a quantitative nature. In general, this theory postulates that viscosity in solution increases proportionally with increasing chain length, and *vice versa*, and that in solution the single chain molecules ("macro-molecules," "thread molecules")^{96a} exist. It is important that the concentration of cellulose in cuprammonium solution be very low so that the macro-molecules do not interfere with each other. The upper limit of concentration, which may be admitted for high polymeric substances in general, is 0.1–0.2 per cent.

The relation between the molecular weight M and the specific viscosity η_{sp} is expressed in the equation:

$$\frac{\eta_{sp}}{C_{gm}} = K_m \cdot M$$

K_m is a constant characteristic for each polymeric series; C_{gm} is the concentration of the solution in basic moles per liter. Specific viscosity represents the increase in viscosity which a dissolved substance produces in the solvent.

Numerous determinations have been carried out according to this method. Staudinger reports good agreement between molecular weights obtained with the viscosity method and those resulting from the osmotic-pressure method.⁹⁷ On the basis of recent determinations⁹⁸ a molecular weight of 324,000 is given for native (cotton) cellulose, of 38,000 to 112,000 for cellulose triacetate, and of 54,000 to 410,000 for nitrocellulose. Again these figures represent average values, for, as mentioned above, the derivatives contain different chain lengths, and so, very likely, does native cellulose from manifold sources and even that from the same source.

^{95a} For the isothermal distillation method see Frazer and Patrick, *Z. physik. Chem.*, **130**, 691 (1927); Frazer, "Colloid Symposium Monograph," **7**, 259 (1930).

⁹⁶ Staudinger and Heuer, *Ber.*, **63**, 222 (1930); Staudinger, *Z. physik. Chem.*, **153**, 391 (1931); *Ber.*, **65**, 267 (1932); Staudinger, "Die hochmolekularen organischen Verbindungen," p. 56.

^{96a} See, however, McBain and McBain, *J. Am. Chem. Soc.*, **59**, 342 (1937); see, also, Hess and Philippoff, *Ber.*, **70**, 639 (1937); Philippoff, *Ber.*, **70**, 827 (1937).

⁹⁷ Staudinger and Schulz, *Ber.*, **68**, 2320 (1935); Schulz, *Z. physik. Chem.*, **176**, 323 (1936).

⁹⁸ Staudinger, *Angew. Chem.*, **49**, 804 (1936).

It is now claimed that the only absolute method for molecular-weight determination of cellulose and cellulose derivatives that yields a correct average value is the ultracentrifugal method originally developed by Svedberg.⁹⁹

This method which has given rather reliable results on proteins is, in addition, capable of supplying directly information regarding the homogeneity of the preparation investigated. It is based upon photographic observations and records of equilibrium or velocity sedimentation in a strong centrifugal field (up to 150,000 revolutions per minute). The values found are much higher than those obtained with the other methods. The following figures are given: 570,000 for native cellulose (corresponding to 3,600 glucose units); 150,000 to 500,000 for purified cellulose, 50,000 to 120,000 for regenerated cellulose, and 45,000 to 100,000 for cellulose acetate. Kraemer and Lansing assume that the values obtained represent the molecular weights of the cellulose preparations under investigation with as great a certainty as those obtainable by the classical methods applied to substances of low molecular weight.

THE FINE STRUCTURE OF CELLULOSE AS REVEALED BY X-RAY ANALYSIS

As mentioned above, x-ray analysis (p. 1758) furnishes definite proof of the crystalline nature of cellulose. It appears that most of the investigators ascribe the lattice on which the cellulose crystal is built to the monoclinic system, with dimensions of the basic cell, i.e., the smallest unit which still possesses the geometrical properties of the whole crystal lattice, expressed in Å ($1 \text{ Å} = 10^{-8} \text{ cm.} = 0.1\text{m}\mu$), as follows (Meyer-Mark-Andress values):^{99a}

<i>a</i> (horizontal)	8.35
<i>b</i> (vertical, representing the length of the basic cell, parallel to the fiber axis)	10.3
<i>c</i> (forming the angle with <i>a</i>)	7.9
$\beta = 78^\circ$	

These dimensions being known, the volume of the unit cell may be calculated, and from the volume and the mass of the anhydroglucose formula unit and the density of cellulose, it was found that four glucose anhydrides may be placed within the unit cell.

Making use of the work of W. H. Bragg and his numerous collaborators on the radii of atoms and the distance between atoms of homo-

⁹⁹ Kraemer and Lansing, *J. Phys. Chem.*, **39**, 153 (1935); Kraemer, lecture, American Chemical Society meeting, New York, April, 1935; Chapel Hill, April, 1937; Lansing and Kraemer, *J. Am. Chem. Soc.*, **57**, 1369 (1935). See, also, Ulmann, *Cellulosechem.*, **16**, 114 (1936).

^{99a} Reference 4e, p. 138.

polar compounds, Sponsler and Dore¹⁰⁰ devised a picture of the possible structural arrangement of the glucose units in the basic cell.

It is most interesting that a decision with regard to the glucose structure to be selected for the arrangement within the unit cell could be made from three-dimensional models carefully constructed to a scale based upon the atomic radii of carbon and oxygen and the distances C—C and C—O. It was found that the amylene oxide ring structure which, as a result of chemical evidence, had been suggested by Haworth, fits best into Sponsler's lattice spacing as derived from x-ray data. For reasons of symmetry, the beta structure was given preference to the alpha structure.

As regards the mode of linking between neighboring glucose units of the chain, Sponsler and Dore erroneously chose alternating glucosidic and ether linkages instead of glucosidic linkages only. However, this has practically no bearing as far as the principles of their conception of the arrangement of the glucose units within the basic cell are concerned. The most essential result was that the investigators recognized the recurrency period (*b*) of 10.25 Å along the fiber axis, as evidenced by x-ray investigations, to be a figure dependent upon the chemical structure of the cellulose. The diameter of one glucose unit, using Haworth's pyranose ring structure, was calculated to be 5.13 Å, that is, just half of the recurrency period. This means that in the unit cell, within the spacing of 10.25 Å on each chain, there occur two glucose units (Fig. 9).*

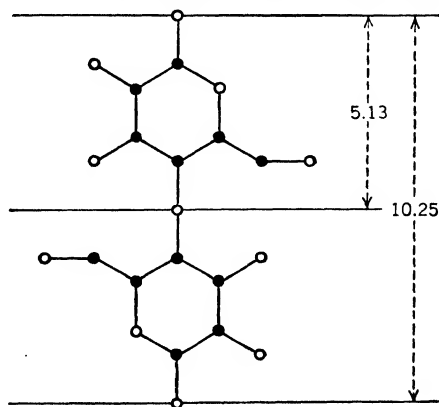


Fig. 9†

¹⁰⁰ Sponsler and Dore, "Colloid Symposium Monograph" [IV], p. 174 (1926); see, also, Sponsler, *Am. J. Botany*, **9**, 471 (1922).

* In this and the following figures, the hydrogen atoms are omitted.

† From Sponsler and Dore, in "Colloid Symposium Monograph" [IV], The Chemical Catalog Co. (1926). (Courtesy of the publishers.)

chains, which run parallel to the fiber axis through the unit cell. The position of the chains with respect to each other, stabilized by secondary valency forces, is shown in Fig. 10.

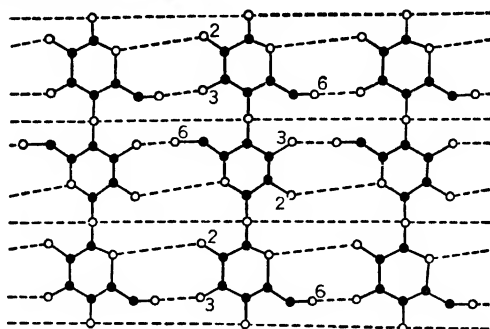


FIG. 10*

Sponsler and Dore's structure would explain a number of the physical phenomena dealt with in previous sections. It would account, for instance, for the swelling in water or in other liquids not attacking the cellulose chemically. This swelling is small in the longitudinal direction of the chains since there is apparently no opportunity for the molecules of these liquids to penetrate between the single units of the chain. In the lateral direction, however, molecules of the liquid find sufficient space to enter and in so doing widen the space still further. This theory is in agreement with the x-ray pattern of the swollen (mercerized) cellulose.¹⁰¹

This structure would account for the courses of the chemical reactions which cellulose undergoes. Thus, the fact that fibrous structure is retained on acetylation, methylation, etc., may be explained by assuming that the new groups insert themselves into the spaces between the longitudinal chains. This will occur more easily the smaller the groups (a conception which is well supported by Trillat¹⁰²), but the linkages of the longitudinal chains and the skeleton of the fiber are not destroyed.

It must be emphasized that Sponsler and Dore's most interesting work has given great impetus to the studies in the years which followed their presentation. Notably, K. H. Meyer,¹⁰³ and Meyer and Mark¹⁰⁴

* From Sponsler and Dore, in "Colloid Symposium Monograph" [IV], The Chemical Catalog Co. (1926). (Courtesy of the publishers.)

¹⁰¹ See, also, Katz, in Hess, "Die Chemie der Cellulose"; also, *Trans. Faraday Soc.*, **29**, 279 (1933).

¹⁰² Trillat, *Compt. rend.*, **197**, 1616 (1933); Trillat and Motz, *ibid.*, **198**, 2147 (1934).

¹⁰³ Meyer, *Z. angew. Chem.*, **41**, 935 (1928).

¹⁰⁴ Meyer and Mark, *Ber.*, **61**, 593 (1928); Meyer and Mark, "Der Aufbau der hochpolymeren organischen Naturstoffe, Akad. Verlags-Ges., Leipzig (1930), pp. 93, 113.

have devoted much thought and experimental study to the problems. Their endeavors were facilitated by the ever-growing accumulation of chemical evidence on questions of constitution, particularly the establishment of the cellobiose formula by Haworth and the abundance of x-ray data gathered from the study of numerous organic compounds.

Meyer and Mark followed the same procedure as that of Sponsler and Dore. They constructed three-dimensional models of the constituent units from balls having multiples of the atomic radii and distances that had been established on other compounds. They also gave consideration to the tendency of the carbon atom to arrange neighboring carbon atoms tetrahedrally around itself.

Further help was derived from Staudinger's ideas on the structure of polymers as well as from investigations by Muller and Shearer,¹⁰⁵ and others of the Bragg school, on long-chain fatty acids. Later, Meyer and Brill¹⁰⁶ obtained confirmation from well-built crystals of lauric acid in which the molecules were found to lie parallel to each other, and the atoms in the chains were found to be arranged in zigzag form.

In their endeavors to accommodate the glucose units in a basic cell, Meyer and Mark used the dimensions as established in 1921 by Polanyi. Further calculations were based upon the β -form of Haworth's cellobiose formula. This may be represented as shown in Fig. 11.* For the construction of this model, 1.54 Å was chosen as the distance between

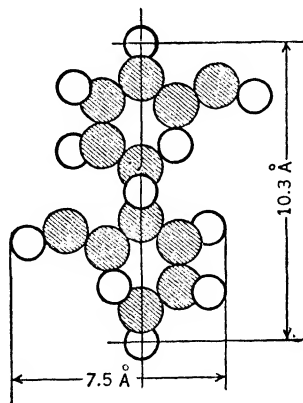


FIG. 11 †

¹⁰⁵ Muller and Shearer, *J. Chem. Soc.*, **123**, 3156 (1923); Muller, *Proc. Roy. Soc. (London)*, [A] **114**, 542 (1927).

¹⁰⁶ Meyer and Brill, *Z. Krist.*, **67**, 570 (1928).

* The shaded circles indicate the carbon atoms; oxygen atoms are represented by the other circles; hydrogen atoms are omitted.

† From Meyer and Mark, "Aufbau der hochpolymeren organischen Naturstoffe," Akad. Verlags-Ges., Leipzig (1930). (Courtesy of the publishers.)

carbon atoms and 1.35 \AA as the distance between carbon and oxygen atoms.

By turning the lower part of the model through 180° and shifting it upward it will cover exactly the upper part of the model. Thus the cellobiose configuration reveals the principle of a diagonal screw arrangement. X-ray analysis has shown that the same principle prevails parallel to the fiber axis and that the screw component equals half the recurrency pattern, that is, 5.13 \AA .

Since the length of the cellobiose model measures 10.25 \AA , it is evident that this length is almost identical with that of the recurrency pattern.

Combining the various pieces of evidence, Meyer and Mark concluded that in the basic cell the cellobiose residues lie parallel to the b axis. Their arrangement is shown in Fig. 12.*

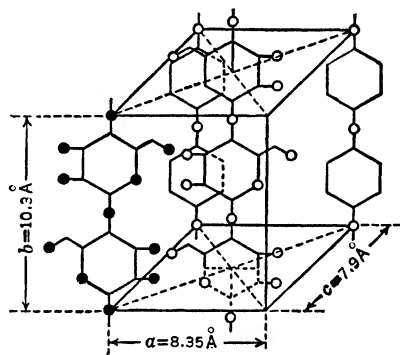


FIG. 12†

An approximate idea regarding the size and form of the micellae or crystallites has been derived from the breadth of the hyperbolas (layer lines) of the diagram. Accordingly, the micella of the ramie fiber is calculated to be a rhombus which measures about 600 \AA along the fiber axis and 50 by 50 \AA across this direction. This estimate due to R. O. Herzog was confirmed by G. L. Clark.¹⁰⁷ One micella would accommodate 1500 to 2000 glucose units, and, assuming the chain to contain 200 glucose units, one micella would comprise ten or less chains. Since in native cellulose the chains are very likely much longer, the number of chains in one micella would, accordingly, be fewer.

* W. Bragg in a letter to *Nature*, **125**, 634 (1930), pointed out that Meyer and Mark's cell and that of Sponsler and Dore are mathematically identical, the side of one cell being the diagonal of the other, and *vice versa*.

† From Meyer and Mark, "Aufbau der hochpolymeren organischen Naturstoffe," *Akad. Verlags-Ges.*, Leipzig (1930). (Courtesy of the publishers.)

¹⁰⁷ Clark, *Ind. Eng. Chem.*, **22**, 474 (1930).

The micellae in the native-cellulose fibers are all oriented parallel to the fiber axis: in cotton, turned spirally around the axis,¹⁰⁸ while in artificial fibers and in films this orientation is missing unless it is produced by stretching. The flax fiber shows the highest degree of orientation.¹⁰⁹ The orientation of the micellae is directly related to the strength of the fibers.

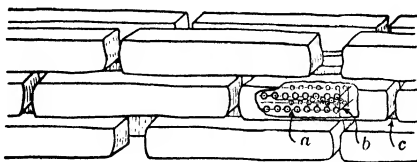


FIG. 13 *

An idea concerning the possible arrangement of the micellae may be derived from a model¹¹⁰ as shown in Fig. 13. Here (a) indicates primary valences between the glucose anhydrides, (b) secondary forces



FIG. 14 †

holding the chains in bundles, and (c) "tertiary" or "micellar" forces between the micellae.

An x-ray diagram of cellulose is shown in Fig. 14.

¹⁰⁸ Farr and Clark, *Contrib. Boyce Thompson Inst.*, **4**, 273 (1932); see, also, Steinberger, *Textile Research*, **4**, 495, 531 (1934).

¹⁰⁹ Morey, *Textile Research*, **4**, 491 (1934).

¹¹⁰ Clark, "Applied X-rays," 2nd ed., McGraw-Hill, New York (1932). A similar arrangement is shown in Hawley and Wise, "Chemistry of Wood," American Chemical Society Monograph, Chemical Catalog Co., New York (1926), p. 26.

* From Clark, "Applied X-Rays," 2nd ed., McGraw-Hill Book Co., New York (1932). (Courtesy of the publishers.)

† Reproduced through the courtesy of Professor G. L. Clark.

THE MICROSTRUCTURE OF CELLULOSE

As regards the relationship which exists between the fibrillae (fiber elements which with certainty may be seen under the microscope) and the micellae on the one hand, and the fiber on the other, an idea may be derived from Lüdtké's work.¹¹¹ The dermatosomes, so termed first by Wiesner (1886),¹¹² and probably identical with Ritter's "fusiform bodies" or "spherical units,"¹¹³ are very small fibrillar sections still discernible under the microscope. They measure 0.5μ in length and $0.3\text{--}0.5\mu$ in diameter. On the basis of the figure for the length of the micella as given by R. O. Herzog and Krüger, one dermatosome would contain 8 micellae, united in a row. A row of 20–100 dermatosomes would make up one fibrilla of $0.3\text{--}0.5\mu$ diameter and $15\text{--}50\mu$ in length. Lüdtké assumes the fibrillae to be united to "striae" and the striae to compose the layers which are cylindrically arranged around the lumen. Finally, according to his theory, the layers are divided up lengthwise into fiber sections by transverse elements, and the fiber sections, about 10 to 100 put together, make up the fiber.

It is interesting to note that no fibrillar structure exists in artificial fibers, although they, like the native fibers, are of micellar structure. This seems to justify Lüdtké's differentiation between the fibrillae as "biological" and the micellae as "chemical" units.

Wiesner obtained his dermatosomes by treating the cell membrane with various reagents such as dilute hydrochloric acid. There is probably some connection between Wiesner's dermatosomes, Ritter's fusiform or spherical units, Hess's crystals, and Wanda Farr's cellulose particles,¹¹⁴ which she isolated from young cotton cell membranes by means of weak acid or alkaline solutions, finding treatment with dilute hydrochloric acid (sp. gr. 1.19) for about 18 hours at room temperature most useful.

The particles, on x-ray analysis, gave a typical Debye-Scherrer diagram and showed by other tests also that their essential cellulose nature was unaltered. Their approximate size was found to be 1.5 by 1.1μ .

From the filtrate of the hydrochloric acid solution in which the cellulose particles are suspended, a colloidal material may be obtained by

¹¹¹ Lüdtké, *Biochem. Z.*, **233**, 1 (1931).

¹¹² Wiesner *et al.*, "Die Rohstoffe des Pflanzenreiches," 4th ed., Engelmann, Dresden (1927), Vol. I, p. 396.

¹¹³ Ritter and Chidester, *Paper Trade J.*, **87**, 131 (1928); Ritter and Seborg, *Ind. Eng. Chem.*, **22**, 1329 (1930); see, also, Hess and Schultze, *Ann.*, **456**, 55 (1927).

¹¹⁴ Farr and Eckerson, *Contrib. Boyce Thompson Inst.*, **6**, 189, 309 (1934); Farr and Sisson, *ibid.*, **6**, 315 (1934); Farr, *Textile Research*, **6**, 518 (1936); Farr, paper presented before the Cellulose Division of the American Chemical Society at Chapel Hill, N. C., April, 1937.

precipitation with alcohol. It amounts to about 3 or 4 per cent of the cell membrane. Its composition is not yet known, but pectic acid is said to be an important part of it. The colloidal substance is termed cementing material and is assumed by Farr to hold the cellulose particles together in the cell membrane.

It appears possible that this cementing material is a part of the so-called "Kittsubstanz" for whose existence in the cellulose fiber O. Herzog believed he had evidence from x-ray analysis, and which has been widely discussed ever since. There also seems to be a connection between the cementing substance of Farr and the so-called "Fremdstanz" of Lüttke which, the latter declares, holds the dermatosomes together.¹¹⁵

From further observations Farr advances the hypothesis that the cellulose particles account for the crystalline behavior, and the cementing substance for the colloidal behavior, of the cell membrane, and she suggests that in many reactions to which cellulose is subjected, at least in their earlier stages, the membrane rather than the cellulose is disintegrated.¹¹⁶

Regarding the formation of the cell membrane from the protoplasm, Farr, in her microscopic studies, finds the cellulose particles in the interior of the young cell scattered throughout the viscous protoplasm, separate or in beadlike chains. In later stages of cell development Farr describes the beadlike chains as made up of single rows of particles, joined end to end, united in layers to form the membrane.

In contrast to the observations of Lüttke and of Farr, I. W. Bailey cannot find any evidence, either in untreated or in carefully swollen fibers, of discrete entities of cellulose, that is, of fibrillae, dermatosomes, or the like, which may be liberated simply by dissolving the non-cellulosic constituent. Such units seem rather to be heterogeneous fragments that are shredded or disrupted from an originally continuous and coherent cellulose matrix. Any discontinuities in the structural pattern of the cellulose are confined to the submicroscopic field, i.e., to the realm of micellae or molecular chains.¹¹⁷

In connection with her studies on cell walls, Farr believes to have evidence for the conclusion that the viscosity of cellulose in cuprammonium solution is due to the cementing substance and not to the cellulose particles. Whereas the cementing substance dissolves, the cellulose particles are said to remain merely dispersed in the cuprammonium solution.

¹¹⁵ Lüttke, *Ann.*, **466**, 27 (1928).

¹¹⁶ Farr, *Textile Research*, **7**, 66 (1936).

¹¹⁷ Bailey and Kerr, *J. Arnold Arboretum*, **16**, 273 (1935). See, also, Kerr and Bailey, *ibid.*, **15**, 327 (1934); Bailey, paper presented before the Cellulose Division of the American Chemical Society at Chapel Hill, N. C., April, 1937; Anderson, *ibid.*; Kerr, *Protoplasma*, **27**, 230 (1937); Anderson and Moore, *Am. J. Botany*, **24**, 503 (1937).

They may be removed from the liquid, and tests show them to be still cellulose.

These observations and the conclusions drawn therefrom are incompatible with the results of all previous investigations, such as those of Hess, Traube, and Staudinger, on the mechanism of the dissolution of cellulose in cuprammonium solution. After exact data are available it may be possible to throw more light on the discrepancies existing at the present time.¹¹⁸

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¹¹⁸ Compton, paper presented before the Cellulose Division of the American Chemical Society at Rochester, N. Y., September, 1937.

CHAPTER 19

MODERN ELECTRONIC CONCEPTS OF VALENCE

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INTRODUCTION

The evolution of valence theories in organic chemistry has been accompanied by the introduction of characteristic symbols that have been applied with considerable success in the interpretation of structural phenomena and of organic chemical reactions. The notion of integral valence bonds and the genesis of structural formulas, together with the recognition of a localized distribution of valence forces in space,

has been sufficient to account in large measure for diverse types of isomerism and in general for the phenomena associated with organic molecules in an inactive or resting state. Efforts to account for dynamic effects in these systems have led to a resolution of integral valence bonds into more or less nebulous components through the assumption of partial valences, primary and secondary valences, and residual fractional polarities. The contribution of these postulates to the growth and general progress of organic chemistry cannot be denied, but efforts to attach a precise physical significance to a valence unit or its hypothetical components had met with insuperable obstacles.

The formulation by Lewis¹ of a more explicit electronic concept of valence and molecular structure has laid the foundation for advances in the direction of expressing chemical affinities in terms of more clearly defined atomic and molecular models. The older theories of organic chemistry and earlier electronic formulations of organic reactions² have taken on more definite form, and a large number of chemical phenomena are seen from a new point of view.

The introduction of the electronic theory and its application to problems of molecular constitution were advanced by the contributions of Langmuir,³ and in the period since 1920 a steady development and elaboration of the fundamental principles of the theory have taken place. Progress in the field of inorganic chemistry has been more rapid than in the organic domain, and many of the important generalizations and correlations have come from studies of the simpler inorganic molecules.

It is evident, however, that the electronic theory must be applicable to chemistry as a whole. A strong impetus to its general acceptance and development was given by Sidgwick,^{4,5} who showed that the underlying principles of the theory and the contributions of modern developments in atomic physics may be used with remarkable success in interpreting the varied chemical behavior of covalent molecules and ionized salts and in elucidating the chemical relations of the elements in the periodic table.

The general application of modern electronic concepts to organic

¹ Lewis, *J. Am. Chem. Soc.*, **38**, 762 (1916); "Valence and the Structure of Atoms and Molecules," Chemical Catalog Co., New York (1923); see, also, *Chem. Rev.*, **1**, 231 (1925); *J. Chem. Phys.*, **1**, 17 (1933).

² Fry, "The Electronic Conception of Valence and the Constitution of Benzene," Longmans, Green and Co., New York (1921). This monograph contains a review of the early applications of electronic concepts to chemical reactions.

³ Langmuir, *J. Am. Chem. Soc.*, **41**, 868, 1543 (1919); **42**, 274 (1920); *Ind. Eng. Chem.*, **12**, 386 (1920).

⁴ Sidgwick, "The Electronic Theory of Valency," Oxford University Press (1929).

⁵ Sidgwick, "The Covalent Link in Chemistry," Cornell University Press, Ithaca (1933); see, also, "Ann. Repts. Chem. Soc. (London), **30**, 110 (1933); **31**, 37 (1934).

chemical reactions is due largely to Robinson⁶ and Ingold⁷ and their collaborators. It must be stated at the outset, however, that this subject is not yet beyond the state of a roughly qualitative solution of the varied and complex problems of organic chemistry, and is in a condition of active development. A good deal of progress has been made and further advances will be forthcoming, especially in the quantitative aspects of the subject.

In 1927 Stewart made this statement:⁸ "The greatest problem before organic chemists at the present day is the application of modern electronic views to the salient phenomena among the reactions of organic compounds. The peculiarities of benzene, the extraordinary variety of effects observed in the rupture of double bonds, and especially the influence of conjugation, are examples of fields which seem to offer outlets for a considerable amount of speculation in connection with G. N. Lewis' theory." At the present time organic chemists are confronted with the serious problem of keeping abreast of the flood of speculative elaborations of electronic theories and seeking to understand and assimilate them.

The Constitution of Atoms. The atom is conceived of as containing a dense nucleus bearing a net positive charge equal to the atomic number of the element. The nucleus is surrounded by a definite number of electrons, sufficient to neutralize exactly the nuclear charge, and these are considered to revolve in concentric orbits or shells (quantum groups). On the basis of physical investigations Bohr⁹ has grouped atoms into four classes: (i) those in which all the shells contain their full complement of electrons (the inert gases); (ii) those in which all but the highest quantum group (outermost shell) are complete; (iii) those in which the two outermost electronic groups are incomplete (the transitional elements); (iv) those in which the three outermost electronic groups are incomplete (the rare earth elements).

The electronic configurations of the inert gases represent maxima of stability. The systems of other atoms tend to approach the stable arrangements of the inert gases by giving up electrons or by acquiring them. The outermost shell (highest quantum group) contains a relatively small number of electrons (less than eight) designated as valence electrons, which are relatively labile. The tendency of these electrons

⁶ Robinson, "Outline of an Electrochemical (Electronic) Theory of the Course of Organic Reactions," Institute of Chemistry of Great Britain and Ireland, London (1932).

⁷ Ingold, *J. Chem. Soc.*, 1120 (1933); *Chem. Rev.*, **15**, 225 (1934).

⁸ Stewart, "Recent Advances in Organic Chemistry," 5th ed., Longmans, Green and Co., New York (1927), Vol. II, p. 354.

⁹ Bohr, "The Theory of Spectra and Atomic Constitution," The University Press, London (1922).

TABLE I

ELECTRONIC CONFIGURATIONS OF THE INERT GASES

Atom and Atomic Number	Electrons in Quantum Groups 1st 2nd 3rd 4th 5th 6th
Helium (2)	2
Neon (10)	2 + 8
Argon (18)	2 + 8 + 8
Krypton (36)	2 + 8 + 18 + 8
Xenon (54)	2 + 8 + 18 + 18 + 8
Radon (86)	2 + 8 + 18 + 32 + 18 + 8

H 1																		He 2							
Li 3																		Be 4	B 5	C 6	N 7	O 8	F 9	Ne 10	
Na 11																		Mg 12	Al 13	Si 14	P 15	S 16	Cl 17	Ar 18	
K 19	Ca 20	Sc 21	Ti 22	V 23	Cr 24	Mn 25	Fe 26	Co 27	Ni 28	Cu 29	Zn 30	Ga 31	Ge 32	As 33	Se 34	Br 35	Kr 36								
← Transitional Elements →																									
Rb 37	Sr 38	Y 39	Zr 40	Nb 41	Mo 42	Tc 43	Ru 44	Rh 45	Pd 46	Ag 47	Cd 48	In 49	Sn 50	Sb 51	Te 52	I 53	Xe 54								
Cs 55	Ba 56	La 57	Ce 58	Pr 59	Yb 70	Lu 71	Hf 72	Ta 73	W 74	Re 75	Os 76	Ir 77	Pt 78	Au 79	Hg 80	Tl 81	Pb 82	Bi 83	Po 84	At 85	Rn 86				
<div style="display: flex; justify-content: space-between;"> — Ra 87 Ac 88 Th 90 Pa 91 U 92 </div>																									

BOHR'S PERIODIC TABLE

BOHR'S PERIODIC TABLE

to form pairs (rule of two) and groups of eight (octet rule) is a basic principle of the theory of chemical combination.

The underlying shells and the nucleus constitute the kernel or effective nucleus of the atom. In hydrogen the kernel is a proton and has a unit positive charge; in the elements of the first short period, from lithium to fluorine, the kernel includes two planetary electrons (the helium pair) and consequently has a net charge, called the effective nuclear charge, of two less than the atomic number. In the elements of the second short period, from sodium to chlorine, the kernel includes ten planetary electrons in two shells (the helium pair + an octet), and the effective nuclear charge is ten less than the atomic number. The atoms most frequently encountered in organic compounds are included in these two periods, in which the number of valence electrons (and the effective nuclear charge) increases regularly from one to seven and corresponds to the group of the element in the periodic table. In general, the atoms with an effective nuclear charge of one or two tend to give up electrons ("electropositive" atoms), and those with a charge greater than two tend to acquire electrons and build up octets ("electronegative" atoms).

Ionic and Covalent Bonds. The union of atoms in a molecule may be effected through two different kinds of interatomic forces, electrovalence or covalence. An electrovalent or ionic link (also called a polar or heteropolar bond) is formed by the complete transfer of an electron from one atom to another, and the binding force is due to electrostatic attraction between the oppositely charged ions. In a covalent link (also called a non-polar or homopolar bond) the union is effected by means of a pair of electrons which is shared by two atoms and is common to the valence shells of both. The two electronic systems interpenetrate, and the atomic nuclei approach each other more closely than in electrovalent bonds. The binding force arising from a shared electron pair is localized and exerted in a definite direction about the atom, whereas the electrostatic attraction of a free ion has no definite direction in space and extends to all ions of opposite sign in its neighborhood.

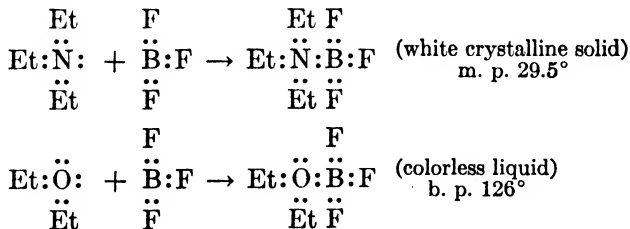
Sugden¹⁰ has developed a theory based upon the hypothesis that atoms may be held together in stable combinations by the formation of a covalent bond in which only one electron is shared by the atomic nuclei, and he has used this assumption to formulate the electronic configurations of a number of inorganic molecules (SF_6 , PF_5 , etc.). There is evidence that hydrogen may form a one-electron covalent bond in the unstable hydrogen molecule-ion $[\text{H}_2]^+$, in diborane and the organoboron hy-

¹⁰ Sugden, "The Parachor and Valency," Routledge and Sons, London (1930).

drides, but it appears on physical grounds that covalent bonds of only one electron may be expected to occur only very exceptionally, and also that a bond of three shared electrons is highly improbable.¹¹ There is certainly no justification for the assumption that covalent bonds of one or three electrons are present in any stable organic molecule.

Coördinate Bonds. Covalent bonds may be considered to arise in two ways: each atom may contribute one electron of the binding pair (normal covalent link) or one of the two atoms may furnish both electrons (coördinate link). A bond of the second type is sometimes called a semi-ionic (semi-polar) or dative double bond. The coördination process involves the union of a *donor* atom possessing an unshared pair of electrons in its valence shell and an *acceptor* atom which is capable of holding two additional electrons. Typical donor atoms include 3-covalent nitrogen in ammonia and amines, 2-covalent oxygen and sulfur, and 1-covalent iodine; examples of acceptor atoms include hydrogen cations (protons), 2-covalent magnesium and zinc in their alkyl derivatives, and 3-covalent boron in its alkyl derivatives and halides.

The union of triethylamine or diethyl ether with boron trifluoride serves to illustrate the formation of a coördinate link. Owing to the fact that nitrogen has five valence electrons it can complete its octet by forming three normal covalent bonds; the resulting 3-covalent nitrogen atom possesses two unshared valence electrons and can act as a donor. Boron has three valence electrons and can acquire but three more by the formation of normal covalent bonds, achieving a total of only six valence electrons. By virtue of the general tendency of a valence sextet to pass into the stable electronic configuration of an octet, 3-covalent boron can acquire two additional electrons and can act as an acceptor. In forming the compound R_3N-BF_3 , the octet of boron is completed by the previously unshared electron pair of the nitrogen.

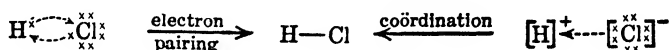


As a result of the coördination process, the acceptor atom obtains a share in two more electrons and its residual positive charge is decreased, while that of the donor atom is increased a corresponding amount.

¹¹ Pauling, *J. Am. Chem. Soc.*, **53**, 3225 (1931); see, also, Chapter 22, p. 1865.

Owing to this disturbance of the systems a coördinate link is sometimes regarded as a double bond made up of one ionic and one covalent bond, or as an intramolecular ion (zwitterion).¹² However, the extent of the sharing of the electron pair by the two atoms of either coördinate or normal covalent bonds is unknown. In both types the residual charges vary over a wide range in different links and probably show time-variability in any given link. In the example of coördination cited above it is sufficient to recognize that the nitrogen atom after coördination is in a condition similar to that in an ammonium ion, and the boron in a state similar to that in a fluoborate ion.

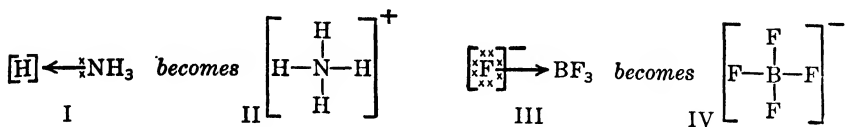
The formation of a coördinate link is denoted conveniently by means of an arrow drawn from the donor to the acceptor, $\text{Et}_3\text{N} \rightarrow \text{BF}_3$, showing the source of the electron pair and the orientation of the resulting dipole ($\overset{\delta+}{\text{N}} \text{---} \overset{\delta-}{\text{B}}$). This symbol refers merely to the manner of establishing the bond and does not imply that the electron pair of a coördinate covalent link differs in itself from that of a normal covalent link. In general the distinction between normal and coördinate covalent bonds vanishes once the bond is established and serves mainly to aid in recognizing a condition in which the covalence of an atom exceeds the number of electrons it can contribute in the formation of the links or the number required to complete a stable group of eight (two, for hydrogen), and the higher covalent state is not accompanied by the appearance of definite ionic charges. The combination of neutral hydrogen and chlorine atoms by electron-pairing produces a molecule of hydrogen chloride that cannot be distinguished from one formed by the coördination of a proton and a chloride ion.



If one of the participants in the coördination is a univalent ion the integral charge is dissipated and the use of a distinctive symbol in the product is superfluous. When a molecule of ammonia undergoes coördination with a proton, the unit positive charge of the proton is distributed throughout the resulting ammonium ion and the new covalence becomes identical with a normal covalence. Likewise, the coördination of boron trifluoride with a fluoride ion produces a new anion in which the unit negative charge permeates the entire system and all the fluorine atoms in the fluoborate ion are held by identical covalences. The normal state of ammonium or fluoborate ions is not represented as a

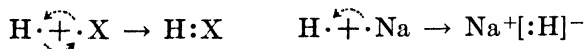
¹² Noyes, *Chem. Rev.*, **17**, 1 (1935).

coördination complex (I, III) but as a system held together by ordinary covalent bonds (II, IV).



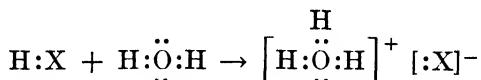
Rule of Covalence Maxima. An expression of the valence of an atom in terms of the electronic theory must take into consideration its capacity to form chemical combinations through electrovalent and covalent linkages. The electrovalence of an atom is determined by the number of its valence electrons; for an atom which tends to acquire electrons it is equal to the number of electrons it requires to attain a stable number, and for an atom which tends to lose electrons it is equal to the number of electrons in excess of a stable number. The normal covalence of an atom is also determined by the number of its valence electrons, but since additional covalent links may be formed by co-ordination, it might appear that the total covalence could vary through wide limits. On the basis of chemical evidence Sidgwick^{4,5} has formulated the following values for the covalence maxima of the elements: **2** (four shared electrons) for hydrogen; **4** (eight shared electrons) for elements of the first short period, from lithium through fluorine; **6** (twelve shared electrons) for elements of the second short period, from sodium through chlorine, and the first long period, from potassium through bromine; **8** (sixteen shared electrons) for atoms of higher atomic number.

Electroaffinity of Hydrogen. It is important to recognize the singular position of hydrogen in the periodic table.¹³ The kernel of hydrogen is a bare proton, and as a result it has a higher effective nuclear charge than any other atomic kernel. The small mass and relatively large charge of the proton account for its extraordinary mobility and for its ability to penetrate the electronic shells of other atoms. Hydrogen acts as a strongly electronegative element through its tendency to acquire an additional electron and attain a stable group of two. It usually forms a single covalent bond but occasionally takes complete possession of an electron pair and forms the hydride anion. The apparent tendency



¹³ Latimer and Rodebush, *J. Am. Chem. Soc.*, **42**, 1419 (1920); Rodebush, *Chem. Rev.*, **5**, 509 (1928); **19**, 59 (1936); see, also, Lowry, *J. Chem. Soc.*, **123**, 822 (1923); Huggins, *J. Org. Chem.*, **1**, 407 (1936); Lassettre, *Chem. Rev.*, **20**, 259 (1937).

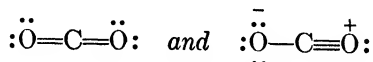
of hydrogen to act as an electropositive atom, as in the ionization of acids, is due to its great mobility. The concentration of *free* protons in the aqueous solution of a strong acid is extremely minute, and the ionization of acids must be regarded as the transfer of a hydrogen nucleus from one molecule to another, thus forming a complex ion.¹³



There is also good physical and chemical evidence that an atom of hydrogen can hold two other atoms together, as in the bifluoride ion, $[\text{F-H-F}]^-$. The association of hydroxylic compounds, and formation of chelate rings (p. 1637) in certain *ortho*-substituted phenols and enolic forms of β -diketones, are further examples of 2-covalent hydrogen. Originally it was assumed that 2-covalent hydrogen held a group of four shared electrons, but this conception has been shown to be quite unlikely on physical grounds. The 2-covalent state of hydrogen is attributed to a condition of resonance between two structures, in the first of which the hydrogen is attached to one, and in the second to the other, of the two atoms which it holds together: $\text{A}:\text{H}:\text{B} \rightleftharpoons \text{A}:\text{H}:\text{B}$.

Resonance (p. 1857). The phenomenon of resonance arises when it is possible for a molecule to have two electronic structures with very nearly the same energy content.¹⁴ In this case neither electronic formula alone expresses the normal state of the molecule but instead a combination of both, with one perhaps more important than the other. The molecule can be regarded as having a structure intermediate between the two (which cannot be expressed by the symbols of structural chemistry) and as achieving stability greater than that of either formula. As a result of resonance the molecule will show the properties of both structures, but in different degrees, and the form with the larger energy content will have the greater influence.

In molecules that exhibit resonance the observed heat of formation is greater than the sum calculated from the heats of formation of the separate bonds, and the increased stability is interpreted as "resonance energy." It is found also that the distances between the atoms linked in a resonating system are somewhat smaller than the normal. These effects may be illustrated with carbon dioxide,¹⁵ for which two different electronic formulas can be written.



¹⁴ Pauling and collaborators, *J. Am. Chem. Soc.*, **53**, 3225 (1931); **54**, 996, 3570 (1932); *J. Chem. Phys.*, **1**, 362, 606, 679, 731 (1933).

¹⁵ Sidgwick, "Ann. Repts. Chem. Soc. (London)," **31**, 37 (1934).

Since the calculated heats of formation of the two forms are nearly the same (348 for the first and *ca.* 350 Cal. per mole for the second), the essential conditions for resonance are satisfied and it should occur. That it does so is indicated by these experimental data: the heat of formation (380 Cal. per mole) is nearly 10 per cent greater than that calculated from the value for a carbonyl group in aldehydes and ketones, and the observed distance between the terminal oxygen atoms is more than 10 per cent less than that calculated for either formula.

The theory of resonance is important in organic chemistry in accounting for the stability of systems which might be expected, on the basis of their structural formulas, to be more reactive than they actually are. Broad generalizations involving this general principle were developed by Ingold,⁷ who has used the concept of tautomeric degeneracy (resonance) and mesomeric effects (resonance effects) with remarkable success in the correlation of molecular structure and chemical reactivity (p. 1680).

The relatively low chemical activity of the carbonyl group of carbon dioxide, in comparison with aldehydes and ketones, offers an illustration of the influence of resonance effects on chemical behavior. Further examples are afforded by benzene (and aromatic systems in general), nitro compounds, carboxylic acids, esters, and anions derived from carboxylic acids or enolic forms of carbonyl compounds (see Table III, p. 1610).

The phenomenon of resonance involves merely a fluctuation of electrons without change of any atomic nucleus and is not to be confused with dynamic isomerism (tautomerism), which requires the displacement of a proton. In resonance, the time of change (if a change is considered to occur) is of the order of 10^{-15} second, but a mixture of two forms in tautomeric equilibrium would change very much more slowly.

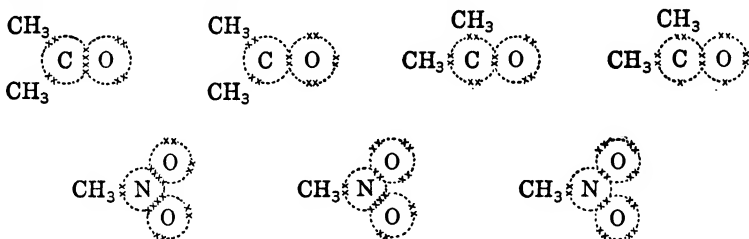
ELECTRONIC CONFIGURATIONS OF ORGANIC MOLECULES

Derivation of Electronic Formulas. The electronic configurations of organic molecules containing only single bonds can be deduced directly from the traditional structural formulas merely by taking into account the number of valence electrons of the atoms concerned, the formation of electron pairs, and the tendency of the principal atoms (C, N, O, S, and the halogens) to form octets. However, double bonds of the conventional formulas may represent two different electronic systems: a true covalent double bond made up of four shared electrons or a coordinate link (semi-ionic double bond) of only two shared electrons.

Where more than one electronic structure is possible, the normal structure may be chosen by using the rule of covalence maxima and the

principle of minimum residual charges formulated by Langmuir:¹⁶ "The residual charge on each atom and on each group of atoms tends to a minimum." For this purpose fractional residual charges arising from an unequal distribution of the electron pair of a covalent bond (inductive displacements, p. 1615) can be neglected and the approximate residual charge is given by the formula, $E - (V_c + V_0)$, where E is the effective nuclear charge of the atom, V_c the number of electron pairs shared with other atoms (covalences), and V_0 is the number of unshared electrons in its valence shell in the compound. In other words, if the sum of the covalences and the unshared electrons for an atom in a compound is greater or less than its effective nuclear charge, the atom bears a residual negative or positive charge.¹²

The use of these rules in deducing electronic formulas may be illustrated with two typical compounds, acetone and nitromethane. In the case of acetone, four possible structures might be considered for the carbonyl group. Since only the first of these satisfies the octet rule and the principle of minimum residual charges, this one may be taken to represent the normal electronic structure. The others may be used to indicate activated states of the molecule arising from dynamic electronic displacements (electromeric effects, p. 1617).



The structure of the nitro compounds offers a more difficult problem. The first formula leaves each atom with no residual charge but violates the octet rule. Although valence shells of more than eight electrons may occur with atoms beyond neon (atomic number, 10) there is strong evidence that the covalence maximum of four is never exceeded in atoms of the first period. In the second formula the octet rule is observed; in this structure the nitrogen has a residual charge of +1, the coordinated oxygen -1, and the 2-covalent oxygen ± 0 . In the third structure the nitrogen has only a sextet of electrons; its residual atomic charge is +2 and that of each oxygen is -1. Although it is possible that nitrogen may sometimes hold a stable group of six electrons, the principle of minimum residual charges indicates that the second formula will represent the normal state. Values of the electric

¹⁶ Langmuir, *Science*, **54**, 59 (1921).

moments of aromatic nitro compounds point to a symmetrical structure for the nitro group, but this can be explained on the basis of a mobile pair of electrons shifting back and forth between two oxygen atoms (resonance). The situation is analogous to the oscillating double bonds in the Kekulé formula for benzene. Problems of electronic configurations in organic molecules have been attacked from the physical side by various means, such as stereochemistry, spectroscopic data, heats of combustion, electric moments,¹⁷ and the parachor.¹⁰

Electronic Symbols. Owing to the complexity of expanded electronic formulas for organic molecules, various conventions have been introduced to simplify the representation of organic structures on the basis of the electronic theory. The usual symbol for an atom is used to designate the atomic kernel, that is, the atom without its valence electrons. The traditional bond of organic chemistry, either expressed or implied, is used with a precise significance to indicate a shared electron pair (covalence). A coördinate link, or semi-ionic double bond, may be shown by changing the covalent bond to an arrow pointing from the electron donor to the acceptor ($A \rightarrow B$) or by using the conventional bond and indicating the resulting charges by plus and minus signs ($A^+ - B^-$).

Unshared electron pairs need not be designated explicitly, since their presence is sufficiently obvious from the number of covalent bonds if the valence shell of the atom is complete. An unpaired electron, in neutral atoms or free radicals, may be indicated by a small dot or by the symbol e ($R\cdot$ or $R-e$). Ions need no symbol beyond the usual $+$ or $-$ sign, but it is frequently convenient to use brackets [] to delimit a polyatomic ion. The use of these conventions is illustrated by means of specific examples in the accompanying table.

Ionic Links. It will be observed that the condensed electronic formulas differ from conventional structural formulas only when ionic or semi-ionic (coördinate) links are present. In the ammonium and diazonium compounds the fact that one of the valences of pentavalent nitrogen is an electrovalence and differs from the other four is well established. Stereochemical evidence¹⁸ (p. 323) shows that the four covalences of nitrogen have a tetrahedral arrangement * but the group

¹⁷ Smyth, "Dielectric Constant and Molecular Structure," Chemical Catalog Co., New York (1931).

¹⁸ Mills and Warren, *J. Chem. Soc.*, **127**, 2507 (1925); see Sidgwick (references 4 and 5) for a general discussion of the space distribution of covalences.

* The tetrahedral arrangement appears to be the only space distribution for elements whose maximum covalence is four, and is the normal arrangement for many 4-covalent atoms of the higher periods (silicon, tin, phosphorus, arsenic, sulfur, selenium, tellurium, etc.). A plane arrangement appears to occur in 4-covalent nickel, palladium, and platinum; an octahedral arrangement occurs in all the 6-covalent compounds that have been examined (aluminum, chromium, iron, cobalt, nickel, platinum, etc.).

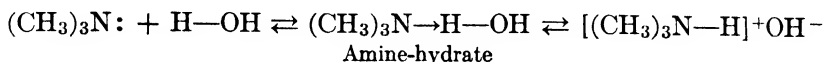
held by the electrovalence does not have a fixed direction in space and is attracted electrostatically by the ammonium ion as a whole.

TABLE II

ELECTRONIC CONFIGURATIONS OF TYPICAL ORGANIC COMPOUNDS

Conventional Formula	Electronic Formula	Condensed Electronic Formula
$\text{CH}_2=\text{CH}_2$	$\begin{array}{c} \text{H} \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad \text{H} \\ \cdot \quad \cdot \quad \cdot \quad \cdot \\ \text{H} \quad \cdot \quad \cdot \quad \cdot \quad \cdot \quad \text{H} \end{array}$	$\text{CH}_2=\text{CH}_2$
$\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3-\text{C}-\text{CH}_3 \end{array}$	$\begin{array}{c} \cdot \quad \cdot \\ \cdot \quad \cdot \\ \text{H}_3\text{C} : \text{C} : \text{CH}_3 \end{array}$	$\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3-\text{C}-\text{CH}_3 \end{array}$
$\begin{array}{c} \text{CH}_3 \quad \text{H} \\ \diagdown \quad \diagup \\ \text{N} \cdots \text{Cl} \\ \diagup \quad \diagdown \\ \text{CH}_3 \quad \text{H} \end{array}$	$\left[\begin{array}{c} \text{CH}_3 \\ \text{H}_3\text{C} : \ddot{\text{N}} : \text{H} \\ \text{H} \end{array} \right]^+ \left[\begin{array}{c} : \ddot{\text{Cl}} : \end{array} \right]^-$	$\left[\begin{array}{c} \text{CH}_3 \\ \text{CH}_3-\text{N}-\text{H} \\ \text{H} \end{array} \right]^+ \text{Cl}^-$
$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3-\text{N}=\text{O} \\ \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \text{H}_3\text{C} : \ddot{\text{N}} : \ddot{\text{O}} : \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_3-\text{N} \rightarrow \text{O} \\ \\ \text{CH}_3 \end{array}$
$\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3-\text{N}=\text{O} \end{array}$	$\begin{array}{c} \cdot \quad \cdot \\ \cdot \quad \cdot \\ \text{H}_3\text{C} : \ddot{\text{N}} : \ddot{\text{O}} : \end{array}$	$\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3-\text{N} \rightarrow \text{O} \end{array}$
$\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3-\text{S}-\text{CH}_3 \\ \parallel \\ \text{O} \end{array}$	$\begin{array}{c} \cdot \quad \cdot \\ \cdot \quad \cdot \\ \text{H}_3\text{C} : \ddot{\text{S}} : \text{CH}_3 \\ \cdot \quad \cdot \\ \cdot \quad \cdot \end{array}$	$\begin{array}{c} \text{O} \\ \parallel \\ \text{CH}_3-\text{S}-\text{CH}_3 \\ \parallel \\ \text{O} \end{array}$
$\begin{array}{c} \text{C}_6\text{H}_5-\text{N}-\text{Cl} \\ \parallel \\ \text{N} \end{array}$	$\left[\text{C}_6\text{H}_5 : \ddot{\text{N}} : \ddot{\text{N}} : \right]^+ \left[\begin{array}{c} : \ddot{\text{Cl}} : \end{array} \right]^-$	$[\text{C}_6\text{H}_5-\text{N} \equiv \text{N}]^+ \text{Cl}^-$
$\begin{array}{c} \text{C}_6\text{H}_5 \\ \\ \text{C}_6\text{H}_5-\text{C} \cdots \\ \\ \text{C}_6\text{H}_5 \end{array}$	$\begin{array}{c} \text{C}_6\text{H}_5 \\ \text{C}_6\text{H}_5 : \ddot{\text{C}} \cdot \\ \text{C}_6\text{H}_5 \end{array}$	$\begin{array}{c} \text{C}_6\text{H}_5 \\ \\ \text{C}_6\text{H}_5-\text{C} \cdot \\ \\ \text{C}_6\text{H}_5 \end{array}$
$\text{Na}-\text{CH}_2-\text{C}_6\text{H}_5$	$\text{Na}^+ \left[\begin{array}{c} \text{H} \\ \cdot \quad \cdot \\ \cdot \quad \cdot \\ : \ddot{\text{C}} : \text{C}_6\text{H}_5 \\ \cdot \quad \cdot \\ \text{H} \end{array} \right]^-$	$\text{Na}^+ [\text{CH}_2-\text{C}_6\text{H}_5]^-$

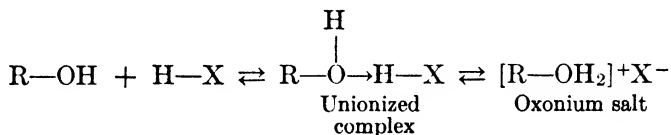
Since nitrogen cannot exceed a covalence of four it might be inferred that an ammonium base or salt could not exist in an undissociated form. This inference is correct only for the quaternary ammonium compounds, since an undissociated form can arise by the formation of a coördinate link involving 2-covalent hydrogen.¹³ An unionized amine-hydrate may be produced by coördination of a covalent hydrogen of water with



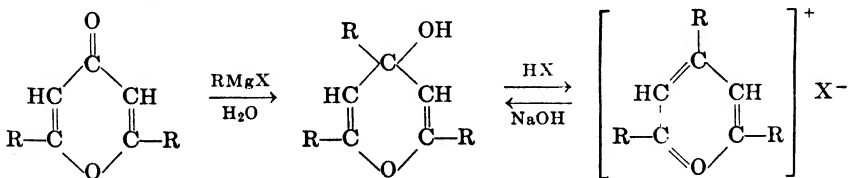
the nitrogen atom of the amine. The stability of the undissociated form is due to the resonance effect associated with a hydrogen bond.*

The tertiary phosphines, arsines, and stibines give rise to quaternary cations strictly analogous to the ammonium cation. In the oxonium and sulfonium compounds, oxygen and sulfur have a covalence of three and one electrovalence; no compounds are known with more than three organic groups attached to oxygen or sulfur. Corresponding cations derived from the halogens occur only with iodine, in the diaryliodonium salts $[\text{C}_6\text{H}_5-\text{I}-\text{C}_6\text{H}_5]^+\text{X}^-$.

Oxonium salts of the type $[\text{ROH}_2]^+\text{X}^-$ and $[\text{R}_2\text{OH}]^+\text{X}^-$ are much less stable than the corresponding ammonium compounds. The interaction of an alcohol and a halogen acid can be represented by the following equilibrium, which is analogous to that of an amine and water:



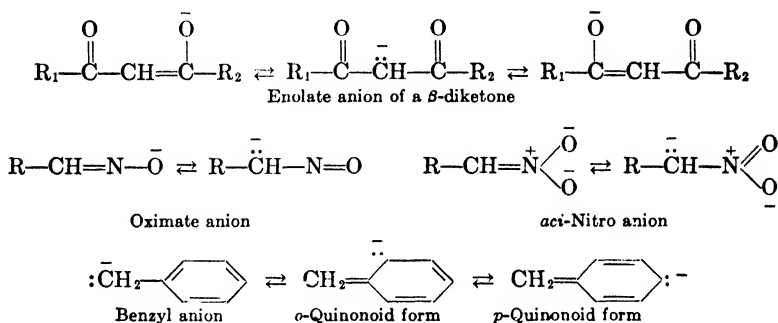
The most stable types of oxonium compounds are the cyclic structures derived from the pyrones. The enhanced stability of the pyrylium and pyroxonium salts may be attributed to an ability of the conjugated unsaturated system to dissipate the high residual charge on the oxygen atom by means of resonance effects.



* The term "hydrogen bond" has been used frequently to indicate the unique bonding of 2-covalent hydrogen, but this expression is ambiguous and the term "hydrogen-bridge" seems preferable; see Huggins, *J. Org. Chem.*, **1**, 409 (1936).

Organic anions occur commonly in the alkali metal salts of carboxylic acids, phenols and enolic forms of β -dicarbonyl compounds, oximes, and *aci*-nitro compounds, and in the organometallic compounds of the alkali metals. It will be observed that all these compounds give rise to structures in which the stability of the anion may be increased through resonance effects, and that the formation of simple ionized salts is re-

TABLE III
RESONATING STRUCTURES OF ORGANIC ANIONS



stricted largely to the alkali metals. Organic derivatives of the less active metals (such as beryllium, magnesium, and zinc) show a marked tendency to form coördination complexes of the Grignard type or to produce chelate ring structures (p. 1637) by intramolecular coördination.

Expanded Valence Shells. Although valence shells of groups of ten or twelve electrons may occur with elements of higher atomic number, experimental evidence indicates that the elements lying between helium and neon cannot expand their valence shells beyond an octet. The hypothesis that the elements in the first period do not expand their valence octets, even in the transitory coördination complexes that arise in the course of chemical reactions, is of considerable value in correlating the reactivity of atoms with their position in the periodic table.

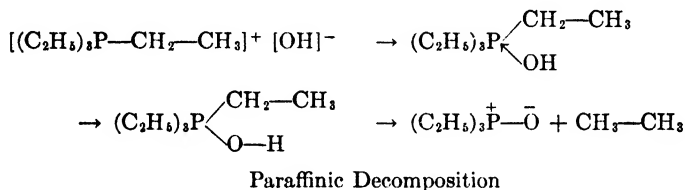
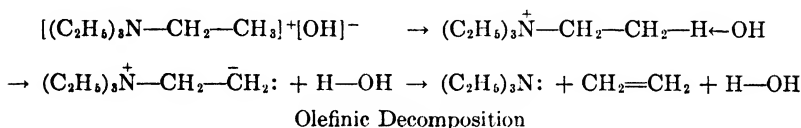
All efforts to obtain derivatives of 5-covalent nitrogen have been unsuccessful. Nitrogen compounds containing five hydrocarbon groups, such as tetramethylammonium benzyl, were prepared by Schlenk¹⁹ and found to behave as ionized salts, $[(\text{CH}_3)_4\text{N}]^+[:\text{R}]^-$ (p. 444). Attempts to obtain compounds with five simple alkyl groups attached to nitrogen, by the interaction of quaternary ammonium halides and metal alkyls, were also fruitless.²⁰ The products of the reactions indicate that the

¹⁹ Schlenk and Holtz, *Ber.*, **49**, 603 (1916); **50**, 274 (1917).

²⁰ Marvel and collaborators, *J. Am. Chem. Soc.*, **48**, 2689 (1926); **49**, 2323 (1927); **51**, 3496 (1929); **52**, 376 (1930).

alkyl group derived from the metal alkyl does not enter the valence shell of the nitrogen atom. Similar experiments with quaternary halides of the phosphonium and arsonium type indicate that even these atoms do not expand their valence shells beyond an octet to hold a fifth alkyl group (pp. 351-52).²⁰ However, the existence of 5-covalent halides of the type R_3PCl_2 and $RAsCl_4$ shows that these atoms can hold a group of ten electrons (decet) when attached to highly electronegative elements.

The marked difference in the mode of decomposition of the quaternary ammonium bases²¹ from that of the corresponding phosphonium, arsonium, and stibonium bases can be accounted for by the assumption that nitrogen is unable to hold a decet of electrons, even as an unstable intermediate state in the course of reaction.

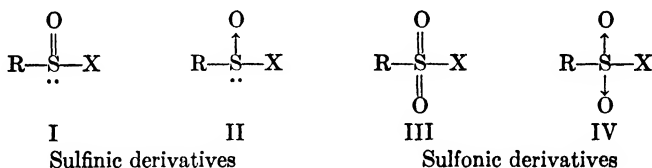


In a quaternary ammonium base the 4-covalent nitrogen cannot furnish a seat for the donor reagent (hydroxyl ion), and the attack occurs through acceptor activity conferred upon a hydrogen atom in the *beta*-position of one of the alkyl groups. Elimination of water leaves the carbon in the *beta*-position with an unshared electron pair and a high residual negative charge; the unshared electron pair is drawn toward the center of high positive charge, and the system breaks up into two more stable configurations, a tertiary amine and an olefin. In the quaternary phosphonium base the central atom is able, by expanding its valence shell, to act as an acceptor for the hydroxyl ion. Subsequent transformations of this complex result from the tendency of the central atom to revert to an octet. Expulsion of the hydroxyl ion merely reverses the original coördination, but the combination of an incipient alkyl anion with a proton from the hydroxyl group within the complex gives an irreversible decomposition into the tertiary phosphine-oxide and a paraffin.

Typical elements of the sixth group (sulfur, selenium, and tellurium)

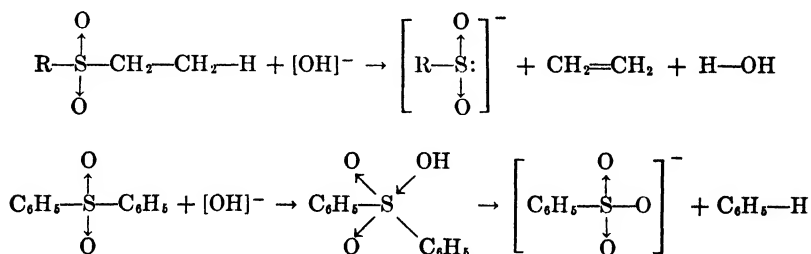
²¹ Ingold and collaborators, *J. Chem. Soc.*, 997 (1927); 3125, 3127 (1928); 2338, 2342 (1929); 705, 708, 713 (1930). Cf. "Ann. Repts. Chem. Soc. (London)," **27**, 143 (1930).

form ionized salts of the type $[R_3S:]^+X^-$ and show little tendency to expand the valence shell to a group of ten. In general, the formation of an expanded valence shell occurs more readily with atoms of higher atomic number, and a group of twelve appears to be more stable than a decet. Tellurium, for example, forms a complex anion $[CH_3-\ddot{Te}I_4]^-$, in which it has five covalent bonds and an unshared electron pair. Although an expanded valence shell of ten or twelve electrons might occur in organic derivatives of sulfur (sulfinic acids, sulfoxides, sulfonic acids, sulfones, etc.), there is definite evidence from measurements of para-



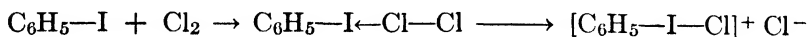
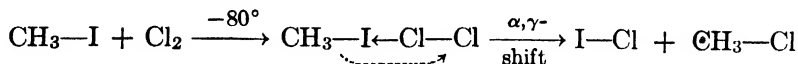
chors¹⁰ and dipole moments that the octet structures (II and IV) represent the true configurations in these compounds.

The cleavage of sulfones by alkalis²¹ gives evidence of the reluctance of sulfur to expand its valence shell, but indicates that it can do so under favorable conditions. The dialkyl sulfones yield an olefin and an alkyl sulfinate; this reaction indicates the direct removal of a proton from the *beta*-position and is strictly analogous to the decomposition of quaternary ammonium hydroxides. In the diaryl sulfones the olefinic decomposition is inhibited and the reaction is analogous to that of quaternary phosphonium hydroxides, which involves a temporary expansion of the valence shell.



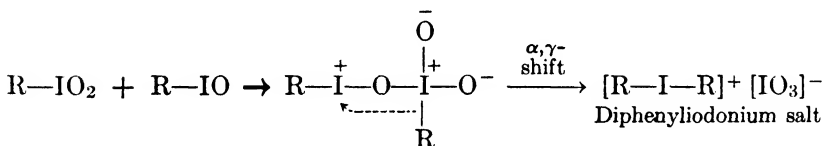
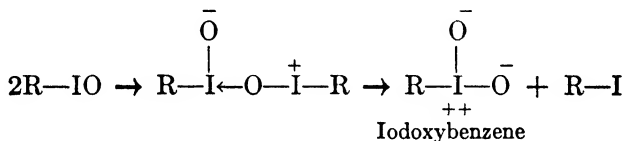
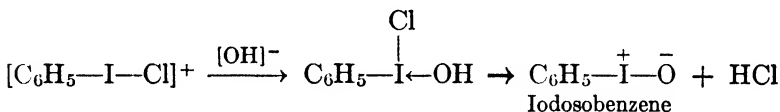
Covalent organic halides in which a halogen atom exerts a covalence greater than one appear to occur only with iodine, and particularly in the aryl iodides. At low temperatures methyl iodide forms a solid dichloride, CH_3-ICl_2 , which decomposes into methyl chloride and iodine monochloride on warming to -30° . The unsaturated alkyl

iodides and aryl iodides form much more stable dichlorides, and the aryl



compounds yield a series of derivatives containing 2-covalent iodine (iodosobenzene and diphenyliodonium salts) and 3-covalent iodine (iodoxybenzene).

The formation of the 2- and 3-covalent iodine compounds and their unusual reactions* can be interpreted upon the assumption that iodine in the link I-Aryl is capable of holding temporarily a decet of electrons but shows a strong tendency to revert to an octet. The structures of the stable derivatives involve only valence octets. A similar mechanism



may be applied to certain reactions of the alkyl halides, and particularly to anomalous reactions of the iodides, since the tendency to expand the valence shell follows the sequence: iodine > bromine > chlorine.

CLASSIFICATION OF ELECTRON DISPLACEMENTS

In the development of current theories dealing with the electronic mechanism of organic reactions by Robinson⁶ and by Ingold,⁷ the activation of a molecule is considered to arise largely through active or incipient electron displacements leading to the development of a center of high or low electron density. Chemical change is pictured as an

* For a discussion of reactions of the iodoxy group (—IO₂) see Masson, Race, and Pounder, *J. Chem. Soc.*, 1669 (1935).

electrical transaction, and molecules are considered to react by virtue of a constitutional affinity either for electrons (electrophiles) or for atomic nuclei (nucleophiles). When an electron-seeking reagent attacks some center in an organic molecule, reaction will take place if the center is able to supply electrons to the requisite extent; the development of a critical electron density at the site of reaction is an essential feature of the development of the energy of activation. Thus, the mechanism of supplying electrons to the reaction zone becomes the main consideration; the more readily the necessary electron density can be furnished, the more the reaction will be facilitated. For a nucleophilic reagent the primary necessity is a center of low electron density at the site of reaction, and groups which withdraw electrons from the reaction zone will facilitate the reaction.

The molecular model which serves as a basis for the modern electrochemical (electronic) theories of reactions is one that visualizes a space distribution of atomic nuclei and electrons (as point charges) maintained by elastic forces about fixed relative positions.⁷ This simple picture has been elaborated, to the dismay and confusion of many organic chemists, by the introduction of wave mechanical ideas of a continuous statistical distribution of electron density, of quantized states, and of resonance (degeneracy). However, the more complicated picture has served, on the whole, merely to correlate and place upon a more definite physical basis a variety of phenomena that were long recognized by organic chemists.

The simpler or the more complex picture leads to the view that the electrical specification of a molecule requires a knowledge of two kinds of electrical quantities. These are concerned with the positions and the mobility of the charges, that is, with the state of polarization of the system *and* with its polarizability. Polarizability represents an intrinsic susceptibility to polarization, a deformability, which becomes operative under the influence of the environment.

The extent to which a given group in an organic molecule can contribute to the activation for reaction involves considerations of the polarization and the polarizability of the group, and of the electrical requirements for the particular reaction. The general acceptance of electronic theories by organic chemists has been delayed by the use of ill-defined conceptions of "polarity," "electron attraction," and "relative electronegativities" (p. 850) along with an insufficient appreciation of the duplex mechanism of activation and of the contribution of the environment (reagents, solvent media, catalysts, etc.). The circumstance that various reactions, intended to measure relative "polarity" or "electronegativity," do not place groups in an identical sequence is

to be expected: polarization and polarizability are independent variables, and their relative contributions vary with the nature of the reaction and of the environment.⁷

The activation of a molecule is considered to involve two forms of electron displacement. Inductive displacements, designated as *I* effects, arise mainly from an unequal extent of sharing of the electron pair of a covalent bond and affect the state of polarization of the atoms in the link; these effects represent a relatively permanent condition of the molecule. Electromeric (dynamic) displacements, designated as *T* or *E* effects, are associated with unshared electron pairs or multiple covalent bonds and concern primarily the polarizability of the structure; electromeric effects are much more time-variable than inductive effects.

Ingold's elaboration of the theory has led to the postulation of four polar effects, as indicated in the following scheme:

Electronic Mechanism *	Electrical Classification	
	Polarization (permanent)	Polarizability (dynamic)
General inductive (<i>I</i>) symbol \rightarrow	Inductive (<i>I_s</i>)	Inductomeric (<i>I_d</i>)
Tautomeric (<i>T</i>) symbol \curvearrowright	Mesomeric (<i>M</i>)	Electromeric (<i>E</i>)

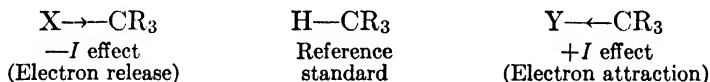
* The notations *I_s* and *I_d* were not used by Ingold but are introduced here to avoid ambiguity in referring to this scheme.

In the present state of the knowledge concerning polar effects, the validity of this analysis or its practical utility is not unquestioned. It is convenient, frequently, to indicate the general mechanisms (*I* and *T* effects) without further reference to permanent or dynamic factors.

The following paragraphs are devoted to definitions of the terms used currently in the application of the theory of electron displacements in organic reactions. It is essential to have a clear conception of the precise meaning of the terms, and to recognize the significance and the limitations of the different electronic effects. Furthermore, two different effects may be present in the same bond and they may reinforce or oppose each other. In the system C—OH, there is an inductive displacement toward the hydroxyl group *and* an electromeric effect in the opposite direction. Each effect can act independently and the contribution in a given reaction requires a consideration of several factors, especially, the electrical demand of the reagent.

General Inductive Effects. Lewis pointed out that the electron pair of a covalent bond may be shared by the two atomic kernels in such a way that there is no *permanent* polarization (as in the symmetrical links, $\text{H}_3\text{C}-\text{CH}_3$, $\text{H}_2\text{N}-\text{NH}_2$, and $\text{Cl}-\text{Cl}$), or the binding pair may be shifted toward one atom so as to give that atom a fractional negative charge and the other atom a corresponding positive charge (as in the unsymmetrical bonds, $\text{H}_3\text{C}-\text{NH}_2$, $\text{H}_3\text{C}-\text{OH}$, $\text{H}_3\text{C}-\text{Cl}$). The term inductive effect is used to designate a permanent displacement (polarization) in which the electron pair remains within the valence shell of both atoms. This displacement is restricted by the fundamental principle requiring the maintenance of stable electronic configurations (especially the octet rule) and is not regarded as sufficient *in itself* to produce a reactive molecule. Inductive effects are considered to act largely through enhancing or restraining electromeric effects. Experimental evidence indicates that *I* effects diminish rapidly in a saturated chain and become negligible beyond two or three atoms.

The direction of inductive effects is considered usually in a relative sense *with reference to hydrogen*, that is, from the standpoint of relative influences of substituents in a given system. A group X would be considered to exert an effect of electron release in the compound $\text{X}-\text{CR}_3$ if the electron density in the residue $-\text{CR}_3$ were greater in this compound than in $\text{H}-\text{CR}_3$. Similarly, the group Y is classified as electron-

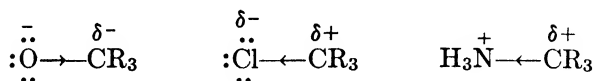


attracting in $\text{Y}-\text{CR}_3$, if the electron density in $-\text{CR}_3$ is less in this compound than in $\text{H}-\text{CR}_3$. Electron release is distinguished by a negative sign and electron attraction by a positive sign, so that they may be indicated by the symbols $-I$ and $+I$ (Robinson).^{*} In structural formulas the direction of electronic displacement may be indicated by an arrow head placed at the *center* of the bond (not to be confused with the symbol for a coordinate link).

Owing to the use of an arbitrary reference standard, an effect of electron release relative to hydrogen does not imply that the group X *necessarily* becomes the positive end of an electrical dipole, nor that the atoms in the link $\text{X} \rightarrow \text{C}$ bear residual atomic charges of opposite sign ("alternating polarity"). Likewise, electron attraction relative to

^{*} In the present discussion the signs attached to *I* and *E* effects are the reverse of those employed by Ingold, although the directions of the effects are considered to be the same. The signs used by Ingold indicate the effect of the displacement upon the groups X or Y, rather than upon $-\text{CR}_3$.

hydrogen does not imply that the group Y becomes the negative end of a dipole. This is shown by a consideration of some specific examples.

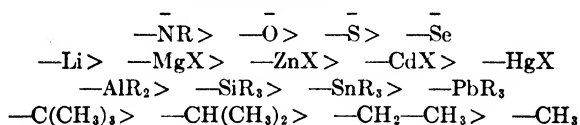


In an alkoxide anion the strong $-I$ effect of the anionic center results in the transfer of a small fractional negative charge (symbol δ^-) to the attached carbon atom, and in the alkylammonium cation the strong $+I$ effect of the cationic center leaves the attached carbon with a small residual positive charge (symbol δ^+).^{*} In both cases the sign of the induced charge is the same as that of the polar group itself. With an electrically neutral substituent such as chlorine, the $+I$ effect of the halogen atom does actually create an electrical dipole, and the atoms in this link bear fractional charges of opposite sign.

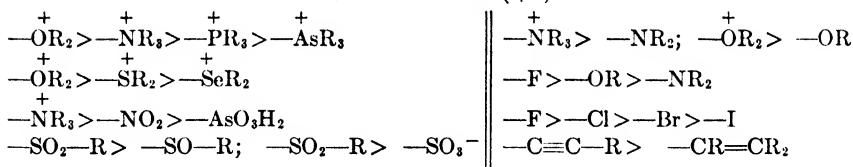
A summary based upon Ingold's classification of the inductive effects of a number of organic groups, and their relative magnitudes, is given in Table IV.

TABLE IV
INDUCTIVE EFFECTS

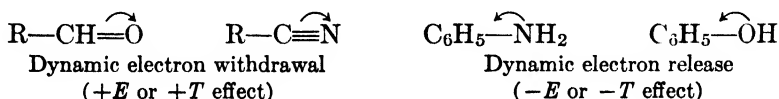
Electron release ($-I$)



Electron attraction ($+I$)



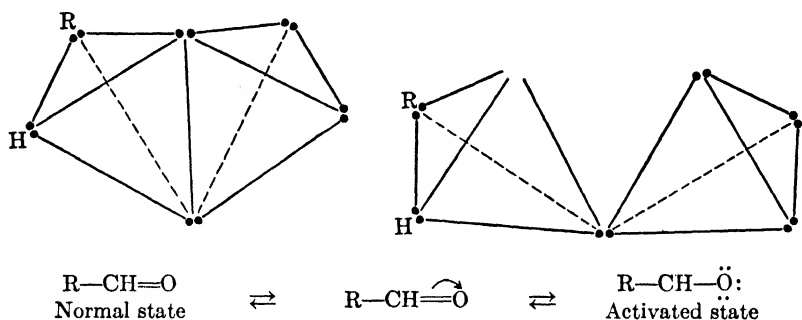
Electromeric Effects. Tautomeric displacements occur in systems containing double bonds or triple bonds, and in single bonds containing an atom that holds an unshared electron pair. In the first case, one



^{*} To avoid confusion the symbols $+$ and $-$ must be restricted to the designation of integral charges of ions or ionic centers. The symbols δ^+ and δ^- are used to indicate fractional charges acquired through electron displacements.

atom tends to withdraw an electron pair from the multiple link and create an electronic deficit in the valence shell of the other atom. In the second, an unshared electron pair is released toward the adjacent atom so that the covalence of the link tends to increase.

The symbol $R-CH=\overset{\curvearrowright}{O}$ implies that to some unknown extent the electrons of the double bond are breaking away from the carbon atom and remaining attached to oxygen. The situation may be pictured from the standpoint of electronic configurations in the following way:



The symbol $C_6H_5-\overset{\curvearrowright}{O}H$ implies that an electron pair of the oxygen atom can be available for the formation of a double bond with the adjacent carbon atom. Owing to the inability of carbon to expand its valence shell, the effect of electron release by oxygen can occur only in the course of a process involving the simultaneous release of an electron pair by the carbon atom. In the case of phenol, the $-T$ effect of the hydroxyl group will facilitate the development of a center of high electron density (donor center), at an *ortho*- or *para*-position of the benzene ring.



Consequently the $-T$ effect will facilitate attack of the aromatic nucleus, at an *ortho*- or *para*-position, by an *electron-seeking reagent*. The $-I$ effect in the link $C-\text{OH}$ does not contribute directly to this kind of reaction since it diminishes the electron density in the aromatic nucleus; it can serve merely to increase the proton-escaping tendency of $C-H$ bonds in the aromatic system. The effects of substituents such as $-\text{NH}_2$ and halogens are similar qualitatively to the $-\text{OH}$ group.

Electromeric displacements of either sign ($+T$ and $-T$ effects) will tend to be restrained by the opposing influence of the electrical

charges that arise concurrently, but other restrictions are more important. Electron withdrawal ($+T$) is held back mainly by the restoring force of the unstable electronic configuration (open sextet) of the deficient atom. Electron release ($-T$) is reversed by the opposing $+T$ effect of the double bond which it creates, but the main consideration is the ability of the attached atom to transmit the effect through one or more multiple bonds (as in phenol or aniline). Even if the attached atom is capable of expanding its valence shell beyond an octet, which is possible for atoms beyond neon (see covalence maxima, p. 1603), the relatively unstable configuration of the expanded shell will have a strong opposing influence. Consequently, electromeric effects represent mainly an inherent ability of the system to undergo an effective electron displacement under the influence of an external polar center.

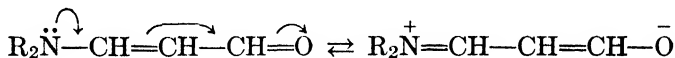
TABLE V
ELECTROMERIC POLARIZABILITIES (INGOLD)

$-E$ (Electron release)				
$-\overset{-}{\text{O}}>$	$-\text{OR}>$	$-\overset{+}{\text{OR}}_2;$	etc.	
$-\text{NR}_2>$	$-\text{OR}>$	$-\text{F};$	etc.	
$-\text{I}>$	$-\text{Br}>$	$-\text{Cl}>$	$-\text{F};$	$-\text{SCN};$ etc.
$+E$ (Electron withdrawal)				
$-\overset{+}{\text{C}}=\text{NR}_2>$	$-\text{C}=\text{NR};$	etc.		
$-\text{C}=\text{O}>$	$-\text{C}=\text{NR};$	etc.		
$-\text{COR}>$	$-\text{COCl}>$	$-\text{CO}_2\text{R}>$	$-\text{CO}-\text{NR}_2>$	$-\overset{-}{\text{CO}}_2;$
$\pm E$ type				
$-\text{C}=\text{C};$	$-\text{C}=\text{C}-\text{C}=\text{C};$	$-\text{C}_6\text{H}_6;$	etc.	

Polarizability effects are concerned with the mobility of the electronic system and are considered to have greater time-variability than inductive effects. Since chemical reaction is assumed to occur only in molecules in an exceptional (activated) state, the momentary surge of electron density associated with the dynamic effects is intimately bound up with the process of activation (p. 1631). The dynamic components (I_d and E) are considered to be capable of giving a direct impetus to one course of reaction but incapable of exerting a *direct* opposition to an alternative course.

Mesomeric Polarization (Resonance Effects). There is physical evidence that molecules containing two appropriately disposed electromeric systems of opposite types form a molecular dipole. The resulting internal compensation of the dynamic effects of electron release and electron withdrawal leads to a diminished activity of the system toward

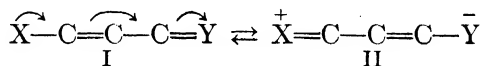
external donor and acceptor reagents. In the system illustrated, the permanent polarization reduces the basic strength of the amino group and depresses the reactivity of the carbonyl group. Robinson, beginning in 1926, developed a number of generalizations pertaining to struc-



tures of this kind, which were classified later as "polyenoid," "heteroenoid," "katio-enoid," and "neutralized" systems.⁶ Examples of these structures and their characteristic behavior are discussed later (Polyfunctional Electromeric Systems, pp. 1679-1698).

A conception of "electronic strain," which is essentially the same as the resonance principle, was adumbrated by C. K. Ingold and E. H. Ingold in 1926. In its subsequent development the terms "mesomerism" and "tautomeric degeneracy" were introduced, and the permanent state of polarization associated with electromeric effects was designated as a mesomeric effect (symbol *M*).

The magnitude of the mesomeric effect in a given system such as (I) will depend upon the relative stability of the alternative structure (II). Mesomeric polarization of (I) requires that X increase its covalence by one unit and acquire a cationic charge, and the opposite change for Y. The presence of preëxisting electrical charges on X or Y

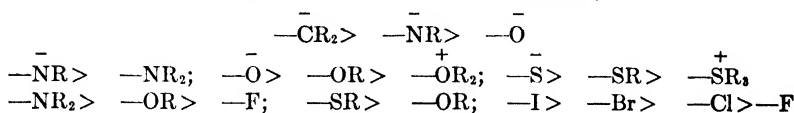


will have an important influence. Electron release by X will be facilitated by a negative charge, and suppressed by a positive charge. Obviously, electron withdrawal by Y will be influenced in the opposite way by electrical charges.

TABLE VI
MESOMERIC EFFECTS

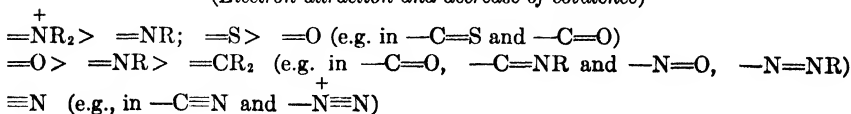
—*M* effect of X

(Electron release and increase of covalence)



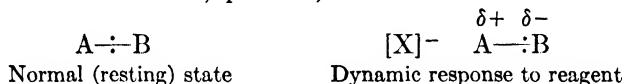
+*M* effect of Y

(Electron attraction and decrease of covalence)



Inductomeric Polarizability. In 1933 Ingold introduced the term inductomeric polarizability to designate a polarizability effect arising in single bonds during the course of reaction by an inductive mechanism, that is, a polarizability effect associated with changes in the sharing of the bonding electron-pair under the influence of a reagent. This concept is the counterpart of the idea of a permanent polarization associated with an electromeric displacement (mesomeric effect).

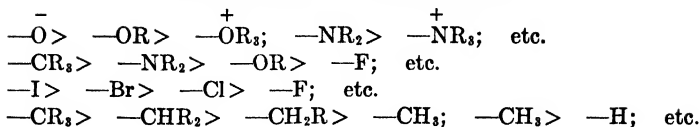
Polarizability effects are due to the deformability of one molecular system under the influence of the polarizing field (polarization) of another. Thus, the close approach of an external polar center may alter the normal distribution of the electron pair of a covalent bond through the deforming action of its polarizing field. The response of a given system to this effect will depend upon the polarizability of its members (see Bond Polarizabilities, p. 1626).



The contribution of inductive polarizability effects is of particular significance in the alkyl groups. These groups merely exert the polar effects which are impressed upon them by other groups in the molecule. The general inductive effect (relative to hydrogen) of CH_3- , and all saturated alkyl groups, is zero if the comparison is made between CH_3-CH_3 and CH_3-H ; but CH_3- exerts a weak effect of electron release ($-I$) if $\text{CH}_3-\text{CO}_2\text{H}$ and $\text{H}-\text{CO}_2\text{H}$, or $\text{CH}_3-\text{C}_6\text{H}_5$ and $\text{H}-\text{C}_6\text{H}_5$, are compared. Since the common organic substituents ($-\text{NH}_2$, $-\text{OH}$, halogens, etc.) have a stronger attraction for electrons than do alkyl groups, the latter will usually exert an effect of electron release. In combinations with groups of lower electron attraction, the opposite effect may be expected.

Alkyl groups are more polarizable than hydrogen, and in the course of reactions one may expect this property to result in a dynamic effect such as $\text{CH}_3 \leftarrow \text{C}$ relative to $\text{H}-\text{C}$. Ingold makes this statement:⁷ "It is provisionally assumed that an inductomeric polarizability is the same for both directions; this would surely hold for small electron displacements, but it is unlikely to be more than roughly true for displacements of the magnitude of those which occur during reactions." Ingold's classification of the inductomeric polarizabilities of typical substituents is presented in the following tabulation.*

* For Fajans' generalizations relating to deformation and deformabilities of ions, see p. 1656. The "deformation rules" of Fajans may be used qualitatively to estimate the relative tendency of covalent bonds to undergo ionization under comparable conditions, and also to estimate the relative stability of organic ions.

\pm INDUCTOMERIC POLARIZABILITIES

It is evident that there are certain special types of organic reactions which cannot be dealt with adequately on the basis of the four electronic effects outlined in the preceding paragraphs. When a reaction involves redistribution of atomic nuclei among themselves (tautomerism, and intramolecular rearrangements), the introduction of additional special principles is required. Furthermore, the two mechanisms of electronic displacement have been considered from the standpoint that all the electrons are paired and cannot be applied without extension to molecules that contain an unpaired electron (free radicals). However, the marked tendency of unpaired electrons to form pairs (rule of two) and the anomalous properties of systems containing an unpaired electron (odd molecules) justify the assumption that the most characteristic reactions of covalent bonds involve retention of the binding pair by one atom of the link rather than fission into free radicals. The formation of free radicals and the interpretation of their chemical behavior will be considered elsewhere (p. 495 and pp. 1698–1704).

POLAR CHARACTERISTICS OF COVALENT BONDS

Residual Charges. In a symmetrical covalent link, A—A or B—B, the binding pair of electrons is distributed equally between the two atomic kernels, so that in the normal or resting state there is no permanent polarization of the link. In an unsymmetrical covalent link A—B, the electron pair may be shifted toward one atom and away from the other, so that A and B acquire fractional charges ($\delta\pm$) and an electrical dipole is created. From estimations of the dipole moments of individual links and of the distances between the atomic nuclei, Sidgwick⁵ has calculated the approximate extent of this displacement, or the inequality of sharing of the electron pair, in a number of the common covalent links. These values are shown in Table VII, where the links are written with the positive end of the dipole at the left, and the symbol $\delta\pm$ expresses the residual charge as a fraction of the charge of an electron (4.77×10^{-10} electrostatic unit).

The fact that atoms of a covalent bond bear fractional charges does not mean that there is no essential distinction between a covalent and an ionic bond, nor does it imply that a covalent molecule A—B exists

TABLE VII
RESIDUAL CHARGES IN COVALENT LINKS (SIDGWICK)

Link $\delta+ \quad \delta- \rightarrow$	Moment ($\Delta E \times d$)	Internuclear Distance d , in Ångström Units	ΔE in Electro- static Units	$\delta \pm$ Electron Charge Units
H—C	0.2	1.14	0.18	0.04
H—N	1.3	1.08	1.2	0.25
H—O	1.6	1.07	1.5	0.31
H—F	1.8	1.0	1.8	0.4
H—P	0.55	1.24	0.44	0.1
H—S	0.8	1.43	0.6	0.13
H—Cl	1.03	1.27	0.81	0.17
H—Br	0.78	1.41	0.55	0.12
H—I	0.38	1.61	0.24	0.05
C—N	0.4	1.48	0.3	0.06
C \equiv N	3.3	1.15	2.9	0.61
C—O	0.9	1.47	0.6	0.13
C=O	2.5	1.27	2.0	0.42
C—S	1.2	1.83	0.7	0.15
C=S	3.0	1.59	1.9	0.40
C—F	1.5	1.45	1.03	0.22
C—Cl	1.7	1.74	1.0	0.21
C—Br	1.6	1.90	0.8	0.17
C—I	1.4	2.12	0.7	0.15
N—O	0.5	1.41	0.4	0.08
N=O	1.9	1.21	1.6	0.34

in equilibrium with the ion pairs $[A^+][B^-]$ and $[A^-][B^+]$. Theoretical considerations and experimental evidence support the view that in the great majority of links the bond will be due almost entirely to electron sharing (covalence), or to electrostatic forces (electrovalence). In typical single covalent links the inequality of sharing of the electron pair does not usually exceed 20 per cent ($\delta \pm 0.2$), and in typical ionic links the reduction in dipole moment resulting from mutual deformation of the ions is usually less than 20 per cent.

It is theoretically possible that in certain rare cases the contributions of the two kinds of valence forces may be nearly equal. If this condition occurred the molecule would be expected to achieve a stability greater than that of either state alone, due to the "resonance energy" of the fluctuation.¹⁴ Unambiguous illustrations of resonance in single bond structures are difficult to find, and in most of the alleged examples other explanations, consistent with the notion of two distinct kinds of bonds, can be adduced.

Bond Energies. Thermochemical data show that an energy change is usually associated with the conversion of two symmetrical covalent molecules A—A and B—B into two unsymmetrical molecules A—B. This change may be considered to parallel the creation of a dipole in the molecule A—B. Pauling has shown that the bond energy of an unsymmetrical molecule A—B, that is, the amount of energy which must be put into the bond to break the gaseous molecule into the gaseous atoms or radicals A· and ·B, is usually larger than half the sum of the bond energies for A—A and B—B. The actual bond energies for unsymmetrical bonds, from experimental determinations, are either equal to or greater than the theoretical values predicted from strict additivity (Table VIII). The difference ΔH , which represents the excess of the

TABLE VIII
RESIDUAL BOND ENERGIES AND RESIDUAL CHARGES
Standard Bond Energies of Symmetrical Bonds (Pauling)
(No residual charges)

Covalent Bond	Bond Energy		Covalent Bond	Bond Energy	
	In Volt Electrons	In Kg. Cal per Mole		In Volt Electrons	In Kg. Cal per Mole
H—H	4.44	102.4	F—F	2.80	64.6
C—C	3.65	84.1	Cl—Cl	2.468	56.90
N—N	1.44	33.2	Br—Br	1.962	45.23
O—O	1.49	34.4	I—I	1.535	35.39

Residual Bond Energies and Charges in Unsymmetrical Bonds

Bond	Bond Energy (in Volt Electrons)		ΔH	ΔZ	$\delta \pm$
	Actual	Predicted			
H—C	4.32	4.04	0.28	0	0.04
H—N	3.89	2.94	0.95	+0.67	0.29
H—O	4.75	2.99	1.76	1.48	0.31
H—F	6.39	3.62	2.77	2.49	0.4
H—Cl	4.38	3.45	0.93	0.65	0.17
H—Br	3.74	3.20	0.54	0.26	0.12
H—I	3.07	2.99	0.08	-0.24	0.05
C—N	2.95	2.55	0.40	+0.12	0.06
C—O	3.55	2.57	0.98	0.70	0.13
C—F	5.40	3.22	2.18	1.90	0.22
C—Cl	3.41	3.06	0.35	0.07	0.21
C—Br	2.83	2.82	0.01	-0.27	0.17
C—I	2.45	2.59	(-0.14)	-0.42	0.15

actual bond energy over the predicted value, will be greater as the residual charge of the bond increases, but there is no simple relationship between the two effects.

Since the H—C link is usually taken as a reference standard for the evaluation of other links in organic molecules, it is of interest to examine the values obtained by subtracting the ΔH for H—C from those for the bonds H—A and C—A. These figures are given in the table under the heading ΔZ , and are placed alongside Sidgwick's values for the residual charges in the links.

An examination of the figures for residual bond energies, or for residual charges, shows clearly that neither represents the relative reactivity of the links and that these data must be used with great care in the interpretation of chemical reactions. Thus, the relative reactivity of the links C—X toward various reagents decreases in the order C—I > C—Br > C—Cl \gg C—F, whereas the residual bond energies or charges decrease in the opposite order. Sidgwick has pointed out that the tendency of hydrogen to become 2-covalent changes in precisely the same way as the residual charges, H—F > H—O > H—N \gg H—C, although it is recognized that this property is influenced by other factors also.

By the use of the ΔH values Pauling has assigned atoms to positions on a map representing relative degree of electronegativity, according to the equation $\Delta_{AB} = (x_A - x_B)^2$, where x_A and x_B represent the coördinates of atoms A and B on the map. The scale of electronegativity as defined in this way is not analogous to the electron affinities of atoms

TABLE IX
COÖRDINATES OF ELEMENTS ON PAULING'S
ELECTRONEGATIVITY SCALE

H	P	I	S	C	Br	Cl	N	O	F
0.00	0.10	0.40	0.43	0.55	0.75	0.94	0.95	1.40	2.00

but is closely akin to the intuitive notion of electronegativity as it is employed by organic chemists, for example, to indicate the relative tendency of atoms or groups to retain the binding pair of electrons when a covalent link is broken in the course of reaction.

Bond Polarizabilities. The rupture of a covalent bond in the course of reaction involves factors other than the extent of polarization in the normal state. The presence of permanent residual charges has an

orienting influence, but the main consideration is the extent to which the atoms of the bond are capable of undergoing a temporary polarization under the influence of polar centers of the reagent, that is, the polarizability of the bond.

The relative polarizability of covalent bonds depends primarily upon the relative *mobility* of electrons within the systems, and can be deduced from refractivities. Since the refraction of light in the visible region is due to the displacement of electrons and not atomic nuclei, it is possible to assign "constants" to groups of electrons rather than to atoms. Detailed analyses of molecular refractions by Fajans and Knoor²² and by Smyth¹⁷ have led to bond refractivities (symbol P_E), and from these values the bond polarizabilities (symbol α) can be calculated. Since the bond refractivities and bond polarizabilities have a linear relationship ($P_E = \frac{4}{3}\pi N\alpha$), the former may be used directly for the comparison of bond polarizabilities. The polarizabilities of some typical bonds are shown in Table X, in which the P_E values given for systems containing unshared electron pairs include the contribution of the unshared pairs.

TABLE X
BOND REFRACTIVITIES

Bond	P_E	Bond	P_E	Bond	P_E
H—C	1.70	C—C	1.21	C=C	4.15
H—N	1.8	C—N	1.55	C≡C	6.02
H—O	1.85	C—O	1.43	C=O	3.42
H—F	1.9	C—F	1.6	F ⁻	2.4
H—Cl	6.67	C—Cl	6.57	Cl ⁻	9.0
H—Br	9.14	C—Br	9.47	Br ⁻	12.6
H—I	13.74	C—I	14.50	I ⁻	19.0

The effect of electrical charges on electron mobility is shown by comparison of the hydrogen halides and the corresponding halide anions; further illustration is afforded by the series [OH]⁻, H₂O, and [H₃O]⁺, where the refractivities are 5.1, 3.76, and 3.0, respectively. The influence of multiple bonds is indicated by comparing C—C with C=C and C≡C; the refractivity of the double bond is 2.075 per electron pair, the triple bond 2.01 per electron pair, and the single bond 1.21. This indicates that the double bond is almost twice as easily polarized as a single bond, but differs very little from a triple bond in its polariz-

²² Fajans and Knoor, *Ber.*, **59**, 256 (1926).

ability.²³ The low polarizability of the single bond C—C accounts also for the rapid diminution of inductive effects in a saturated carbon chain and the greater polarizability of C=C for the ability of unsaturated systems to transmit them with much smaller loss.

The refractivities of the bonds C—X follow the same order as the general reactivities of the halides in metathetical reactions involving elimination of the halide anion. It must be recognized, however, that polarizability effects can occur in either direction, and a high polarizability of systems containing an unshared electron pair may be related to the tendency to form additional covalent links by coordination with an acceptor center. The stability or reactivity of the resulting structures involves a number of other factors and cannot be predicted directly from the relative polarizabilities.

CLASSIFICATION OF CHEMICAL REACTIVITIES

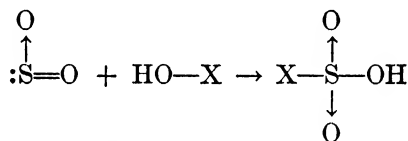
Organic molecules may be classified superficially from the standpoint of the electronic configurations of the valence shells of the principal atoms, but the important consideration in dealing with chemical reactions is the behavior of their active centers relative to other systems. The classification of reagents as acids and bases, or as oxidizing and reducing agents, is merely a convenient expression of their activity relative to one another. Within a given category it is possible to place reagents along a scale of relative affinities to express the activity of acids, or oxidizing agents, relative to each other. Obviously, the possession of one dominant characteristic does not necessarily imply the absence of the opposite property; a base will act as such in contact with a reagent that is less basic than itself but can act as an acid in the presence of a reagent more basic than itself.

Acids and Bases. Lewis¹ has given the following broad definition of basicity and acidity: a basic substance is one which has an unshared pair of electrons which may be used to complete the stable group of another atom, and an acidic substance is one which can employ an unshared pair from another molecule in completing the stable group of one of its own atoms. Thus, acetic acid is acidic with reference to hydrocarbons, alcohols, and amines, all of which are relatively stronger electron donors, but it is basic with reference to hydrogen chloride, which is a weaker electron donor. It is possible to arrange molecules and ions in the order of their acidic or basic activity relative to a definite

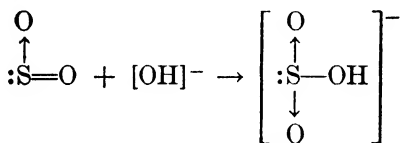
²³ Waters, "Physical Aspects of Organic Chemistry," Routledge and Sons, London (1935).

criterion, but it is not to be expected that the identical sequence will be maintained toward all reagents of either type.

Oxidation and Reduction. A consideration of oxidation and reduction shows that the reducing agent donates electrons, or a share in its electrons, to the oxidizing agent. Consequently, oxidation and reduction are analogous to basicity and acidity from the standpoint of the fundamental electronic characteristics of the active centers. Bases and reducing agents are electron donors; acids and oxidizing agents are electron acceptors. In any category the reagents may be subdivided into two groups, depending upon whether they act by donating or accepting electrons completely or by a sharing process. For example, an active metal or ion ($\text{Na}\cdot$ or Fe^{++}) usually acts as a reducing agent, or as a "base," by donating electrons completely; covalent molecules (SO_2 , NH_3 , etc.) usually act by donating to an oxidizing agent, or to an acid, a share in previously unshared electron pairs. Sulfur dioxide acts as a reducing agent by virtue of the unshared electron pair in the valence shell of the sulfur atom; it can act also as an oxidizing agent or acid, by virtue of its ability to accept an unshared electron pair from another molecule or ion ($[\text{OH}]^-$ or NH_3).



SO_2 as electron donor



SO_2 as electron acceptor

Cationoid and Anionoid Activity. Following Lapworth's proposal that reagents should be classified as anionoid or cationoid according as they resemble active anions or cations in their behavior, Robinson has arranged the active centers of typical reagents into two groups indicating their behavior relative to one another in the course of reactions (Table XI).⁶ It must be emphasized that the terms cationoid and anionoid are not intended to imply that the nature of the electroaffinity depends upon the state of polarization of the reactive system. These names refer to the characteristic acceptor or donor activity of reactive cations or anions.

TABLE XI

CLASSIFICATION OF REAGENTS (LAPWORTH-ROBINSON)

Anionoid (Electron-donating)

Reactive anions: $[\text{NH}_2]^-$, $[\text{OH}]^-$, $[\text{CN}]^-$, $[\text{OR}]^-$, $[\text{CH}(\text{CO}_2\text{Et})_2]^-$, etc.

Molecules containing unshared electron pairs: N of NH_3 and amines; O of H_2O , ethers, aldehydes, ketones; S of mercaptans, sulfides; etc.

Reducing agents: Fe^{++} ; metals (as sources of electrons).

Hydrocarbon residues of organometallic compounds: R— of R—MgX ; $\text{R—C}\equiv\text{C—}$ of acetylides; etc.

Unsaturated carbon of olefins and of aromatic compounds: $\text{CH}_2=\text{CH}_2$; C_6H_6 .

Neutral atoms and free radicals (common to both classes).

Cationoid (Electron-accepting)

Protons and proton sources: acids, etc.

Reactive cations: $[\text{H}_3\text{O}]^+$, diazonium ions, cations of pseudo bases (e.g., cotarnine).

Metallic atoms with incomplete valence shells, capable of coordination: HgR_2 , etc.

Alkyl residues of esters, alkyl halides, and quaternary ammonium compounds:

$(\text{CH}_3)_3\text{SO}_4$, $\text{CH}_3\text{—X}$, $[(\text{CH}_3)_4\text{N}]^+$, etc.

Halogens, ozone, peroxides, and oxidizing agents: CrO_3 , Fe^{+++} , MnO_4 , etc.

Carbon of carbonyl groups (aldehydes, ketones, esters) and nitriles.

Nitrogen of nitroso and nitro compounds, and nitric acid.

Sulfur of SO_3 , H_2SO_4 , NaHSO_3 .

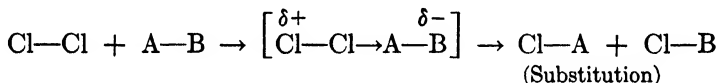
Neutral atoms and free radicals (common to both classes).

Electrophiles and Nucleophiles. Ingold has designated reagents which donate their electrons to, or share them with, a foreign atomic nucleus as nucleophilic; reagents which acquire electrons, or a share in electrons previously belonging to a foreign molecule or ion, are termed electrophilic. The broad classification of organic molecules and reagents as electron acceptors or electrophiles, and electron donors or nucleophiles, embraces also the narrower classifications (acids and bases, oxidizing and reducing agents). It is evident, therefore, that all the methods of classifying the electroaffinities of organic molecules and reagents are based upon essentially the same principles and the same electrochemical concept of chemical activity (p. 1614).

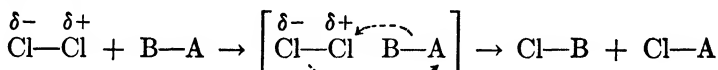
Formulation of Reaction Mechanisms. It must be recognized that the formal classification of reagents as electrophilic and nucleophilic, or cationoid and anionoid, is based upon considerations of the initial and final states of the reactive centers and is independent of specific hypotheses concerning the intimate mechanism of the reaction process. From the standpoint of reaction mechanisms the intrinsic electroaffinity of an active center, or the overall transition from the initial to the final state, is less significant than the nature of the process through which the electronic transfer is accomplished. The formulation of definite reaction mechanisms depends upon the introduction of various speculative

hypotheses designed to correlate the chemical behavior of active centers with their electronic configurations.

A certain confusion arises from the circumstance that the inherent attraction of a reactive center for electrons or for atomic nuclei may operate by a direct or indirect process. There is little doubt, for example, that the driving force of the characteristic reactions of molecular chlorine arises from an inherent attraction for electrons. Ingold regards chlorine as an electron-accepting reagent; it is considered so in the Lapworth-Robinson classification and placed in the same category with metallic atoms having an incomplete valence shell, potential alkyl cations, and the carbon atom of the carbonyl group. However, in speculating upon the mode of attack of reagents by chlorine it appears that the avidity of chlorine for electrons may be appeased by a circuitous process: one atom can act as a donor in a preliminary attachment to an acceptor center and the other atom then migrates to a donor center of the reagent. The result of the whole process is that both chlorine atoms attain a state in which they have a larger share in the binding pair of electrons than either had in the Cl—Cl link.



The same reaction can be formulated by the assumption of a preliminary polarization of the chlorine molecule by the electrical field of a molecule of the reactant, or a solvent, or surface on which adsorption has occurred.



The reaction is then considered to result from the strong electron attraction of the electron-depleted (positively polarized) chlorine atom. The same products would be expected according to either formulation, so that an examination of the final products will not be of assistance in distinguishing between the alternative mechanisms. Indeed, the source of instability is due in both cases to the same active center, the electron deficiency of the positively polarized chlorine atom.

In the following discussion an effort has been made to incorporate the pertinent features of the important generalizations of Robinson and of Ingold, and to present a composite picture of the contributions of a number of other investigators in the application and extension of electronic theories. Since the formulation of intermediate complexes seems justified on physical and chemical grounds and useful in the inter-

pretation of many effects that are associated with reactivities, particular emphasis has been given to the hypothesis of preliminary coordination complexes. The reactive complexes are intended to represent mobile systems which facilitate the occurrence of effective electron displacements in the course of reaction and must not be confused with stable intermediate compounds that can sometimes be isolated.

CLASSIFICATION OF REACTIONS

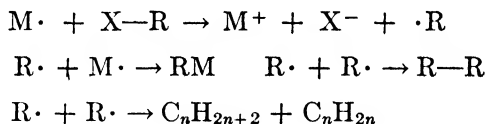
The most typical reactions of organic molecules that occur in solutions and at ordinary temperatures may be grouped into three general classes, according to the nature of the reagent: (i) reactions between a covalent molecule and a free atom, especially an alkali metal, or a free radical; (ii) reactions between a covalent molecule and an ionized substance; (iii) reactions between two covalent molecules. It is by no means a simple matter to decide upon the intimate mechanism of all organic reactions, but it may be stated that, in general, only the first class of reactions will involve a symmetrical fission of the binding pair of electrons to yield an electrically neutral fragment, a free radical. These reactions may, therefore, be designated as non-ionic or radical reactions. The second and third classes may be considered to involve an unsymmetrical cleavage in which the electron pair remains intact and is retained by one of the atoms of the link. Reactions of this kind need not be sharply differentiated, and both are frequently called ionic reactions. It is convenient to designate the second class as simple ionic and the third class as pseudo ionic or complex ionic reactions.

The three classes of reactions of covalent molecules usually differ markedly from the ionic reactions of strong electrolytes in that the former take place more slowly. To account for this difference Arrhenius introduced the notion that chemical reaction depends upon the presence of a relatively small number of active molecules, and that a normal or average molecule must be brought to a higher energy level (activated state) before reaction will occur. This conception has been extremely fruitful, and the Arrhenius equation $k = Ze^{-E/RT}$, relating the reaction velocity k with the number of molecular collisions and the energy of activation E , has served as a basis for the general correlation of kinetics of chemical changes. In the activation theory the quantity E represents the amount of energy which a molecule must have in excess of the normal or average energy content, and, since this quantity enters as an exponential term, small variations in E will produce a relatively large effect upon the rate of reaction.

Physical evidence leads to the view that there is a time interval

between an effective collision and the occurrence of reaction, and that an "activated complex" intervenes as an intermediate state between the reactants and the products. The driving force of a reaction depends upon energy increments that are associated with polarization and polarizability effects in a covalent structure. In the activated complex relatively small forces are able to bring about a redistribution of the electronic systems among the atomic nuclei, to give more stable configurations.

Radical Reactions. The interaction of an organic halide and an alkali metal may be taken for consideration of the general mechanism of this class of reactions. The metal transfers an electron to the halide molecule forming a highly unstable organic anion. The transient anionic complex $[R-X+e]^-$ decomposes into X^- and $R\cdot$. The subsequent transformations will depend upon a number of factors: the intrinsic stability of the free radical, which is a constitutive property determined by internal factors; the nature of the environment; and the relative concentrations of other molecules or atoms with which it can react (external factors). Reaction of the free radical with a second atom of the metal will generate an organometallic compound RM ; reaction with another free radical may produce either $R-R$ (Wurtz-Fittig reaction) or $R-H$ and an olefin (disproportionation). It is possible to attribute the formation of $R-R$ to the interaction of RM



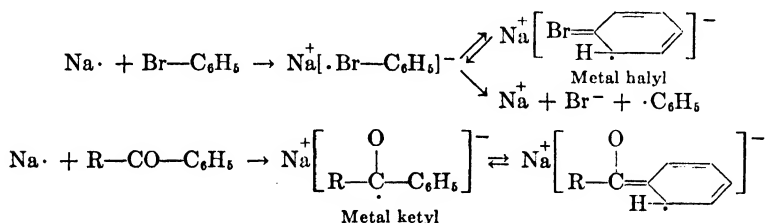
with a second molecule of $R-X$, and to envisage other mechanisms leading to the same products, but these details need not be considered here (see p. 452).

The relative reactivity of a series of halides ($R-F$, $R-Cl$, $R-Br$, and $R-I$) toward a given metal should be determined by the relative ability of a collision with the metallic atom to produce an effective electronic displacement in the direction $X \rightarrow -R$. Consequently the polarizability of the $C-X$ bond, and not the residual charge in the link, will be paramount, and the reactivity should decrease from iodide to fluoride. This is verified in the most striking way by ingenious experiments of Polanyi and his collaborators,²⁴ who have shown that the number of collisions required to produce one effective collision, in the reaction of methyl halides with sodium vapor, is 1.5 for CH_3-I , 25

²⁴ Horn, Polanyi, and Style, *Trans. Faraday Soc.*, **30**, 189 (1934).

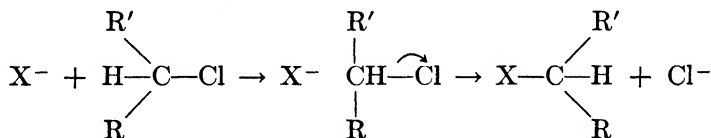
for $\text{CH}_3\text{—Br}$, 5000 for $\text{CH}_3\text{—Cl}$, and 10,000,000 for $\text{CH}_3\text{—F}$. Similar results were obtained with the ethyl and aryl iodides, bromides, and chlorides.

There is some evidence that the attack of sodium on organic halides such as bromobenzene gives rise to transient sodium halyls (p. 454), corresponding to the ketyls obtained from aromatic carbonyl compounds.²⁵ This suggests that the reactions under ordinary conditions probably occur by the addition of an electron to the halogen atom, through the temporary expansion of its valence shell. In aromatic



halyls and ketyls the relatively longer life of the intermediate may be attributed to resonance effects. The halyl complex may be regarded as a temporary state which permits internal dynamic effects to become operative.

Simple Ionic Reactions. The metathetical reactions of alkyl halides with hydroxides, alkoxides, and salts of alkali metals are typical of this class. The rate of reaction usually follows the order $\text{R—I} > \text{R—Br} > \text{R—Cl}$, in accordance with the polarizability of the halogen atoms. The fact that reactions of this kind can occur without racemization of an asymmetric system (p. 1844) attached to the halogen atom, but with optical inversion,²⁶ indicates that the process may be pictured in the following way:



In this case the configuration of the asymmetric center is “turned inside out, like an umbrella in a strong wind.” A preliminary separation of *free* alkyl cations in reactions of this kind is quite improbable, since this would lead to racemization and possibly to molecular rearrangement within the alkyl group.

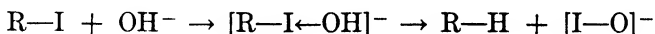
²⁵ Morton and Stevens, *J. Am. Chem. Soc.*, **54**, 1919 (1932); see, also, Bachmann and Wiselogle, *ibid.*, **58**, 1943 (1936).

²⁶ For a discussion of optical inversion, see p. 196 and also p. 1844.

The effect of structural variations of the alkyl group upon the rates of reaction has been studied for many reactions of this type. Although different ionic reagents do not always place groups in an identical sequence, it is generally true that the order of decreasing reactivity is: methyl > primary > secondary > tertiary groups. Aryl and vinyl halides are extremely inert, and allylic or benzylic halides are highly reactive (p. 832).

Since alkyl groups tend to produce an effect of electron-release relative to hydrogen, it might be expected that the tertiary alkyl halides should be more reactive than the secondary or primary compounds. However, the determining factor here appears to be the mobility of the cationic center, that is, the relative tendency of a collision to produce a sufficient mobility of the respective cationic centers. Since the alkyl groups are electron-releasing groups they reduce the electronic deficit and thereby diminish the probability of an effective collision. Aryl and vinyl groups inhibit reaction through their tendency to favor a mesomeric polarization which increases the covalence of the carbon-halogen bond (see hetero-enoid systems, p. 1679). The interposition of a methylene group, as in $\text{CH}_2=\text{CH}-\text{CH}_2-\text{Cl}$ or $\text{C}_6\text{H}_5-\text{CH}_2-\text{Cl}$, has a strong positive effect since the vinyl or aryl system is capable of exerting a strong *dynamic effect* of electron release toward the atom to which it is attached. Mesomeric polarization ($-T$ effect of the halogen atom), which disfavors separation of a halide anion, does not occur in the allyl and benzyl halides owing to the inability of the methylene group to hold an additional electron pair. It will be recalled that dynamic effects are able to facilitate a given type of reaction, but cannot in themselves impede an alternative reaction.

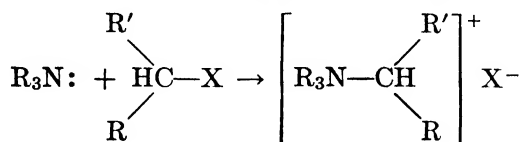
In certain reactions, and especially with elements of high atomic number, the mechanism may involve a temporary expansion of the valence shell beyond an octet (p. 1610). The anomalous hydrolysis of certain halides, particularly iodides, to give hydrocarbons is an example of this phenomenon.



This type of reaction occurs in cases of "positive" halogens, such as $\text{R}-\text{C}\equiv\text{C}-\text{X}$, *p*-amino-aryl halides, certain α -halogenated ketones, etc.

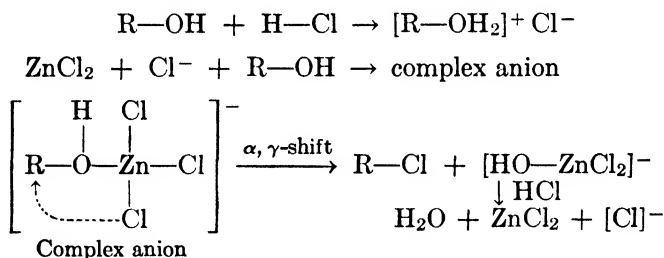
Pseudo Ionic Reactions. The rates and mechanisms of many reactions between covalent molecules are similar to those observed in simple ionic reactions. There is no fundamental distinction between the reaction of an alkyl halide with an amine, and that with an ionized metallic salt. In this type of reaction, also, optical inversion takes place and not racemization. However, it is frequently observed that

reactions between covalent molecules are greatly facilitated by the presence of media or catalysts that are capable of giving rise to coördination complexes.²⁷ This suggests that interchanges between



covalent bonds may occur as the final stage of a mechanism that is initiated by a coördination process.

The formation of unstable coördination complexes will tend to accentuate or diminish polarization effects and will increase the opportunity for dynamic contributions of multiple bonds and unshared electron pairs. The presence of an unstable electronic shell in the complex increases the electron mobility, and the proximity of the reacting molecules about the coördination center allows a suitable approach of covalent systems in the form of loosely bound cations and ions. The effect of zinc chloride in promoting the formation of alkyl halides from primary alcohols and halogen acids may be taken as an illustration. In the absence of zinc chloride, a simple ionization of the halogen acid takes place but the rate of formation of alkyl halide is extremely slow.



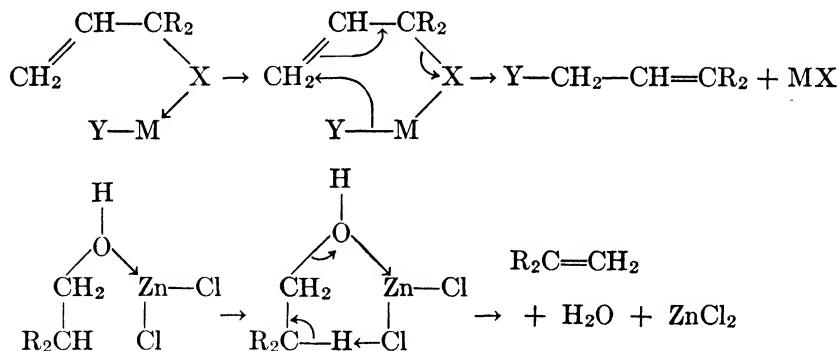
The introduction of zinc chloride makes possible the formation of an unstable coördination complex such as $[\text{R-OHZnCl}_3]^-$, in which an incipient alkyl cation and chloride anion are brought into close contact. By an α, γ -shift within this complex the alkyl halide is produced, and since the latter has little tendency to coördinate with zinc chloride it is liberated from the complex. Action of the halogen acid upon the basic zinc complex regenerates the catalyst and the cycle can be repeated.

The factors influencing the formation and the behavior of coördination complexes are rather obscure, but certain general features can be recog-

²⁷ Meisenheimer and collaborators, *Ann.*, **442**, 180 (1925); Meerwein, *Ann.*, **455**, 227 (1927).

nized. Owing to the circumstance that the transition elements cannot produce cations of an inert gas configuration by simple ionization, these elements show a marked tendency to increase their valence shells by coördination with unshared electron pairs of ions or covalent molecules. Compounds of the transition elements are, therefore, particularly active acceptor centers. Elements in groups II and III of the periodic table also show marked tendencies to form coördination complexes, but the alkali metals do so with much less facility. The relative sizes of the potential donor and acceptor centers, and other steric factors, appear to be of considerable significance also. In general, the formation of *stable* complexes is more likely to occur when the acceptor and donor centers are of approximately the same size.

There is evidence to support the hypothesis that a number of organic reactions, including certain molecular rearrangements and addition reactions, may occur through an ephemeral cyclization within the primary reaction complex by means of a subsequent intramolecular coördination (chelation, p. 1637). The rearrangement of allylic halides,²⁸ certain reactions of Grignard reagents,²⁹ and many other reactions may involve a process analogous to the following:



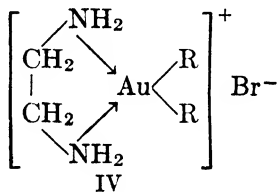
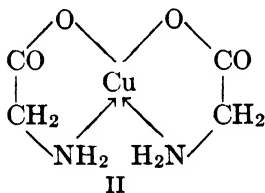
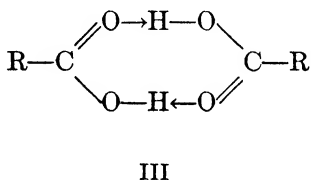
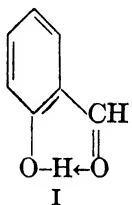
It is difficult to formulate the fugitive chelate complex with the usual valence symbols, and the intermediate cyclic form must be regarded merely as a symbol for the sequences of electron displacement that occur and not as a structural formula. The process corresponds to an electrical circuit that is started by the initial coördination of X with the center M, and is completed by rupture of the bond between X and CR₂. Other examples of cyclic reaction mechanisms are discussed in connection with chelate rings (p. 1649).

²⁸ Carothers and Berchet, *J. Am. Chem. Soc.*, **55**, 2810 (1933).

²⁹ Johnson, *ibid.*, **55**, 3029 (1933).

CHELATE RINGS

The term chelate ring denotes a cyclic structure that arises through intramolecular coordination in systems containing a donor and acceptor center, as in salicylaldehyde (I) and the covalent copper salt of glycine (II), or a ring that is formed by intermolecular coordination in systems that are capable of forming two or more coordinate links. The dimers of the carboxylic acids (III), and a variety of metallic complexes derived from ethylenediamine (IV) or anions of dicarboxylic acids, are repre-



sentatives of the intermolecular type. The name "chelate" is derived from the stem *chela*, a pincer-like claw, and was proposed in 1920 by Morgan and Drew.³⁰ The existence of ring structures in the coordination complexes of ethylenediamine and similar compounds had been known much earlier, but recognition of the importance of the phenomenon of ring closure by coordination and its bearing on chemical problems appears to have been due largely to the work of Morgan and his collaborators.³¹ They showed, for example, that mordant dyes are chelate structures and that the ability to dye cloth mordanted with metallic salts is due to structural features of the dye that permit chelation to occur.

It must be recognized that the process of forming a chelate ring is the same as that leading to the simple open-chain coordination complexes. Indeed, some chelate systems differ very little from the open-chain analogs. However, the chelate rings of special interest in organic chemistry are those in which the cyclization makes possible the occur-

³⁰ Morgan and Drew, *J. Chem. Soc.*, **117**, 1457 (1920).

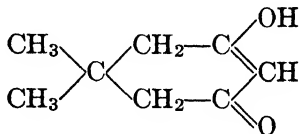
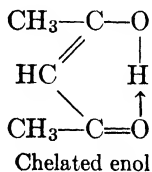
³¹ Morgan and Main Smith, *J. Soc. Dyers Colourists*, **41**, 233 (1925).

rence of resonance effects, or serves to alter preëxisting resonance effects.

The existence of chelate structures cannot be doubted and is supported by experimental evidence derived from stereoisomerism, ionization phenomena, molecular association, solubility behavior, and spectroscopy. Probably the most convincing evidence for the ability of hydrogen to become 2-covalent is afforded by the demonstration that hydrogen can take part in the formation of chelate rings.

In certain *ortho*-substituted phenols the presence of chelate ring structures was postulated by Sidgwick⁴ to account for the fact that whenever the substituent has the structure necessary to form a *six-membered* chelate ring (as —CO—H , —CO—R , —CO—OH , —NO_2 , etc.) the *ortho* isomer differs markedly from the *meta* and *para* in physical properties and is always less highly associated. The *ortho* isomers are less soluble in water, are more soluble in benzene, and have a lower boiling point.

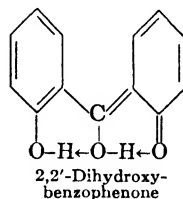
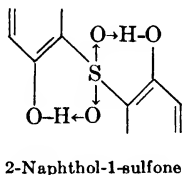
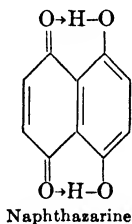
Although much evidence for the existence of chelate rings involving 2-covalent hydrogen has been adduced from considerations of simple physical properties, the most convincing demonstration has come from infra-red spectroscopy.³² It has been found that absorption in the region characteristic of the hydroxyl group ($6200\text{--}7500\text{ cm.}^{-1}$) is absent for a large number of compounds having configurations that would favor formation of a chelate ring containing the hydrogen bond $\text{O—H}\leftarrow\text{O}$ or $\text{O—H}\leftarrow\text{N}$. The characteristic hydroxyl absorption was retained in related compounds where the constitutions or configurations excluded the formation of such bonds. Thus, the characteristic absorption was absent for phenols containing *ortho* substituents such as —CO—H , —CO—CH_3 , —NO_2 , and —CO—NH_2 , but was present in the *meta* and *para* isomers. Absence of hydroxyl absorption (in 0.1–0.03 molar solutions in carbon tetrachloride) was noted for acetylacetone, benzoylacetone, and dibenzoylmethane, but not in compounds where chelation is excluded on steric grounds.



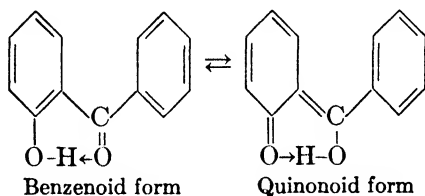
The presence of more than one hydrogen bond within a molecule is shown by the absence of hydroxyl absorption in 1,4- and 1,5-dihydroxy-

³² Hilbert, Wulf, Hendricks, and Liddel, *J. Am. Chem. Soc.*, **58**, 548, 1991 (1936).

anthraquinone, and 1,4-dihydroxy-5,8-naphthoquinone (naphthazarine). Other interesting examples of the formation of two hydrogen bonds are afforded by 2-naphthol-1-sulfone and 2,2'-dihydroxybenzophenone. The former shows also that oxygen atoms held in semi-ionic linkage with sulfur can participate in forming intramolecular hydrogen bonds.³²



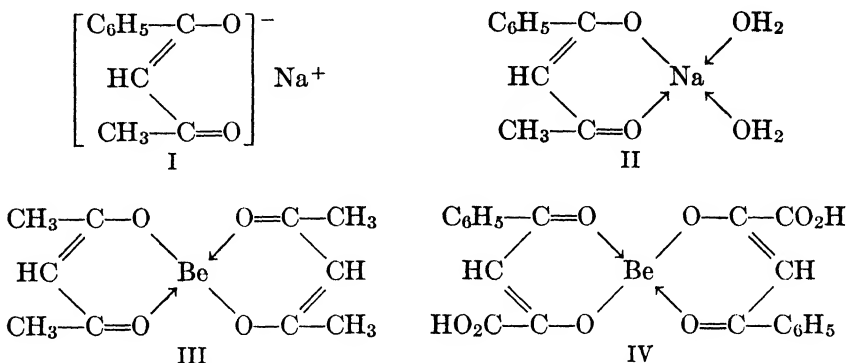
There is evidence that the chelation of hydroxyl groups with *ortho* carbonyl or nitro groups in aromatic systems is accompanied by an increase in color, which may be interpreted as a tendency for the chelation to develop a quinonoid structure within the aromatic ring. The resonating forms of a typical *o*-hydroxyaryl ketone correspond to benzenoid and quinonoid structures, and the color may be considered to be a contribution of the latter. Thus, 2-methoxybenzophenone cannot undergo chelation and is colorless, but 2-hydroxybenzophenone can form a chelate ring and is pale yellow in color. The introduction of an



ortho hydroxyl group into the adjacent ring, as in 2,2'-dihydroxybenzophenone, gives a double chelation that is accompanied by a large increase in quinoidation, and the compound is bright yellow.

Metallic derivatives of the enolic forms of β -ketonic esters, β -diketones, and similar tautomeric systems, may be ionized salts or covalent chelate rings. The anhydrous form of the sodium derivative of benzoylacetone behaves as a typical ionized salt (I) and is insoluble in hydrocarbons, but it forms a dihydrate which is soluble in toluene and is clearly a covalent molecule containing a chelate system (II). Similar covalent dihydrates are formed by the lithium derivative of benzoylacetone and of methyl salicylate, and by the sodium derivative of aceto-

acetic ester. A dichelate system of spirane type is present in the beryllium derivative of acetylacetone (III), and trichelate systems occur in the corresponding aluminum and silicon derivatives.



Convincing evidence for the existence of the chelate structures is afforded by the fact that the copper and beryllium derivatives of benzoylpyruvic acid (IV) have been resolved by Mills and Gotts into optically active forms.³³

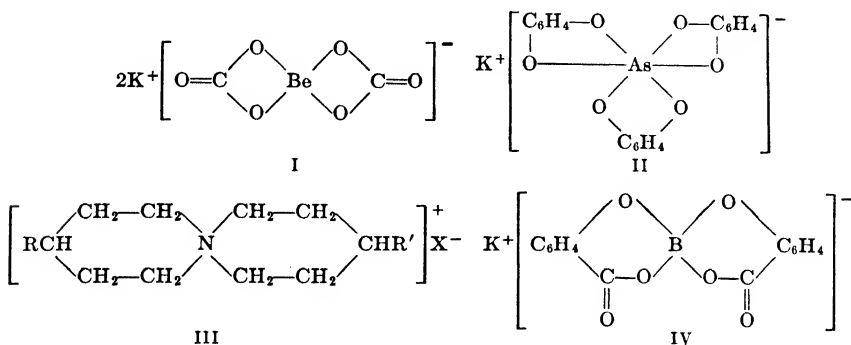
Sidgwick has devised a convenient classification of chelate rings into three types, on the basis of the nature of the bonds that are present in the cyclic system.⁴ Rings in which the coördinate link becomes identical with a normal covalent bond as a result of chelation are designated as type A; those containing one or two definite coördinate links are denoted as types B and C, respectively. The ring types are not always sharply differentiated, and frequently, in the liquid state and in solutions, the chelate systems exist in equilibrium with a non-chelate structure. In some cases electromeric (resonance) effects within the system render the classification doubtful.

Type A. These rings result from the chelation of ions and arise through the inability of the central atom to form additional covalent links except by coördination. They are generally quite stable and, in addition to the usual five- and six-membered systems, may contain cycles of four or seven members which are found rarely in other types of chelate rings.

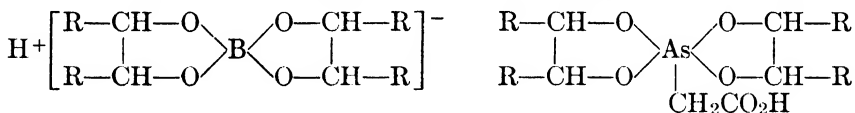
The double carbonates and sulfates of beryllium afford an illustration of four-membered rings of this class (I). Five-membered rings occur in various derivatives of catechol (II); six-membered systems, in the bis-piperidinium salts (III) and in the boro-salicylates (IV). All

³³ Mills and Gotts, *J. Chem. Soc.*, 3121 (1926). See, also, Chapter 3, p. 356.

of these are relatively stable, and the chelate structures of the last three have been confirmed by resolution into optically active forms.



Certain 1,2-glycols form five-membered chelate ring structures in aqueous solutions of boric acid. The effect of the chelation is to produce a large increase in the acidic strength of the boric acid, owing to the enhanced stability of the chelate anion. It has been observed, as might be expected, that the spatial configuration of stereoisomeric 1,2-glycols has an influence upon the tendency to form the chelate structures (p. 396). The *cis*-form of cyclopentane-1,2-diol or hydrindane-1,2-diol is found to increase the acidic strength of boric acid, but the *trans*-form does not; the racemic form of hydrobenzoin has a positive



Chelate esters of boric and arsonoacetic acids

effect, but the *meso*-form is without effect. The steric and constitutional requirements for the chelation are not perfectly clear, since many aliphatic 1,2-diols (ethylene glycol, 1,2-propylene glycol, and pinacol) have no effect on boric acid and in some cases both pairs of optical enantiomorphs, or *cis-trans* isomers, produce the same effect.³⁴ The 1,2-glycols also form chelate structures with arsenic acid and with arsonoacetic acid.³⁵

Cyclic oxonium compounds, such as the pyrylium and pyroxonium salts (p. 1609), may be regarded as a special case of chelate structures of type A. They are analogous to the bis-piperidinium compounds in that the stability of the ring structure is associated with the presence

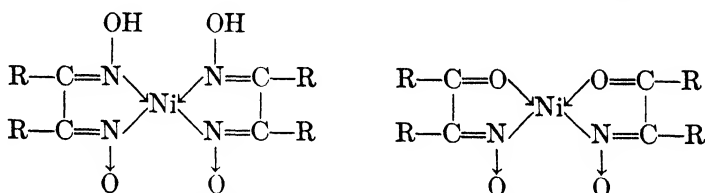
³⁴ Böeseken and collaborators, *Rec. trav. chim.*, **39**, 185 (1920); **40**, 525, 553 (1921); **41**, 327, 722 (1922).

³⁵ Englund, *J. prakt. Chem.*, **122**, 121 (1929).

of a cationic charge produced as the result of a coördination process. With the pyroxonium compounds the ring structure can persist in the absence of an electrical charge, but shows a strong tendency to go over to an open-chain carbonyl compound (under the influence of alkalis).

Type B. In these systems an atom is held in the ring on one side by a normal covalent link and on the other by a coördinate link. The rings are usually less stable than the preceding type and always contain either five or six members. The type B chelate structures most frequently encountered are five-membered rings containing one double bond, and five- or six-membered rings containing two conjugated double bonds. The conjugated rings of this class are probably the most extensive group of chelate structures.

The covalent metallic derivatives of α -ketonic acids, α -amino acids, *o*-nitrosophenols, mono- and dioximes of *o*-quinones and 1,2-diketones (dimethylglyoxime, benzil mono- and dioximes), benzoin oxime, 2-pyridyl ketoximes, and 8-hydroxyquinoline are familiar examples of five-membered rings of type B. The oxime complexes have been for-



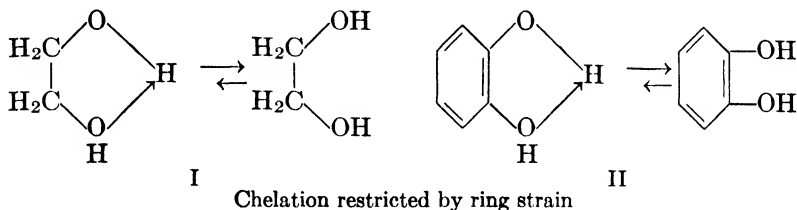
Chelate nickel derivatives of oximes

mulated as six- or seven-membered ring structures, but there is now definite evidence from stereochemistry for the five-membered ring. It is found that the formation of stable metallic complexes occurs only when the configuration is favorable for the structures given above. Thus, complex salts are produced readily from the *anti*-CHOH form of benzoin oxime, the *anti*-2-pyridyl form of 2-pyridyl ketoximes, and the *anti*-form of benzil mono- and dioximes; the *syn*-forms of these oximes do not yield metallic complexes.³⁶

Although the saturated compounds (glycols, amino alcohols, diamines, etc.) that could give rise to rings of this type may undergo chelation to some extent, it appears that intermolecular coördination (association) to form open-chain structures occurs more readily. Saturated five-membered rings of Type C are present in metallic complexes formed from 1,2-glycols, 1,2-amino alcohols, and 1,2-diamines, but there is little evidence to support the view that these substances form Type B chelate

³⁶ Meisenheimer and Theilacker, in Freudenberg's "Stereochemie," Deuticke, Leipzig and Vienna (1933), pp. 1039 ff.

rings involving 2-covalent hydrogen. Crystal structure analysis of a number of compounds indicates that the valence angle of 2-covalent hydrogen is 180° and that a distance of about 2.6 Ångström units for the $O-H\leftarrow O$ or $O-H\leftarrow N$ systems is favorable. The chelate forms of saturated 1,2-diols (I) and related types, and of analogous

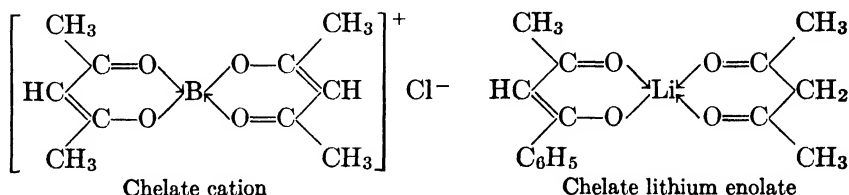


aromatic compounds (II) where a double bond is present, would involve large deviations from the normal valence angles and would therefore be relatively unstable. Evidence from infra-red spectroscopy shows that the characteristic hydroxyl absorption is present in compounds such as catechol and benzoin but other physical properties of catechol and *o*-aminophenols suggest that chelation may occur to some extent.

Saturated 1,3-glycols and β -hydroxy carbonyl compounds fulfill the necessary geometrical considerations for the formation of six-membered rings containing hydrogen bonds. However, it is evident that these conditions alone do not suffice since typical examples of such compounds (β -hydroxybutyraldehyde, and esters of tartaric acid) show absorption in the region characteristic of the hydroxyl group. In these cases the diminished tendency to form intramolecular hydrogen bonds may be attributed to the freedom of rotation about the single bonds and the absence of stabilizing resonance effects that can occur in the corresponding unsaturated types (enol forms of 1,3-diketones, etc.).

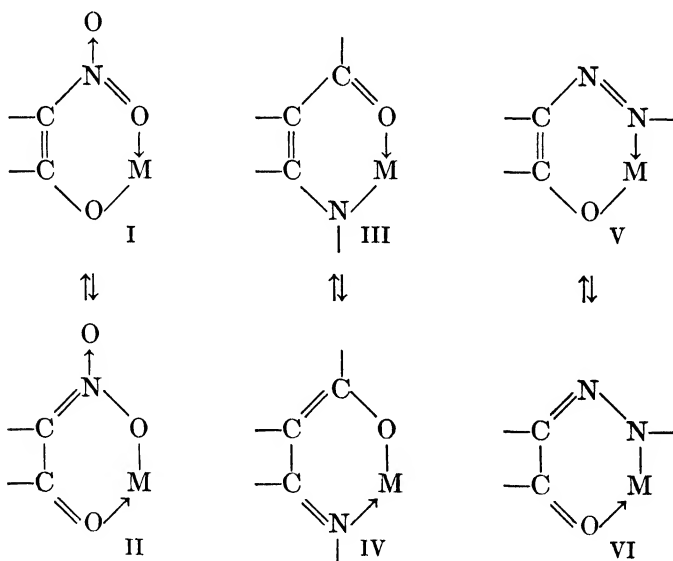
Six-membered rings containing two conjugated double bonds and one coördinate link are very frequently encountered, and are probably the most important chelate rings in organic chemistry. Owing to the favorable steric relations and the intervention of resonance effects, these rings are relatively stable. Either hydrogen (becoming 2-covalent) or a metallic atom acts as the acceptor center, and nitrogen or oxygen acts as the donor atom. Typical examples of chelation through hydrogen are the *o*-substituted phenols (p. 1638) and enolic forms of β -diketones, β -ketonic esters, and other tautomeric systems. Many of the metallic derivatives of these substances form unusually stable covalent chelate structures; the beryllium, aluminum, copper, and certain other metallic derivatives of acetylacetone can be distilled without appreciable decomposition.

Boron and silicon give stable chelate cations with acetonylacetone and similar compounds. The alkali metal derivatives of acetonyl acetone, and of enolic systems in general, are usually open-chain ionized



salts and show little tendency to form chelate structures (lithium > sodium > potassium). However, in these compounds the stability of the chelate form may be increased by further coördination with a molecule of the free diketone or with a solvent, as in the covalent dihydrates already cited (formula II, p. 1640).

A number of six-membered rings of this class contain oxygen and nitrogen, and occasionally sulfur. Several of the typical chelate systems containing nitrogen are shown in the general formulas I-VI, where M may be hydrogen or a metal and one of the double bonds is frequently part of an aromatic structure. Formulas I and II represent

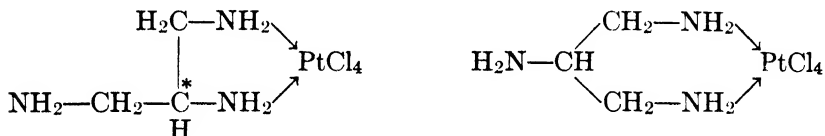


o-nitrophenols; similar types appear to be formed also by *o*-hydroxysul-

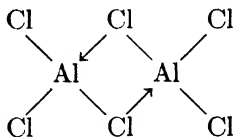
ones. Formulas III and IV include β -aminocrotonic esters, indigo, anils of *o*-hydroxy aromatic aldehydes, and *o*-amino aromatic carbonyl compounds. *o*-Hydroxyazo compounds, hydrazones of α -ketonic esters, and monohydrazones of 1,2-diketones, are examples of formulas V and VI.

Type C. Rings containing two coördinate links are generally the least stable of the three types owing to the fact that relatively stable molecules (or ions) are formed when the ring is broken. The most common examples of this type are encountered in complexes involving a powerful coördination center such as cobalt, nickel, iridium, or platinum. Five- and six-membered rings are found in the complex amines containing ethylenediamine and trimethylenediamine, 1,2,3-triaminopropane, 2,2'-bipyridyl, 2-aminomethylquinolines, etc.

Evidence that the saturated five-membered rings are formed more readily than similar six-membered rings is afforded by the mode of chelation in the compound of platinum chloride and 1,2,3-triaminopropane.



The isomeric five- and six-membered cycles differ in that the former has an asymmetric carbon atom and the latter has not. The product was resolved by Mann and Pope³⁷ and consequently must have a five-membered ring structure.



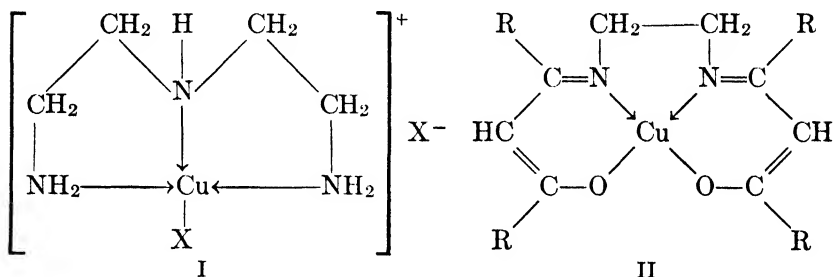
A good deal of evidence supports the view that the dimeric covalent halides of the trivalent metals, such as aluminum and ferric chlorides, are four-membered rings of this type. Since aluminum and iron are able to assume a plane configuration for four covalences the strain in these four-membered chelate structures is reduced. Eight-membered rings containing two coördinate bonds and two double bonds occur in the dimers of the carboxylic acids. Owing to the circumstance that the valence angle of 2-covalent hydrogen is 180°, the symmetrical eight-

³⁷ Mann and Pope, *Nature*, **119**, 351 (1927); Mann, *J. Chem. Soc.*, 1224 (1927).

membered ring (formula III, p. 1637) involves no greater strain than a six-membered ring.³⁸ The chelate structure accounts for the observed low dipole moment and the fact that polymerization does not proceed beyond double molecules.

Lewis and Schutz³⁹ have made the interesting observation that replacement of the acidic hydrogen of acetic acid by its heavier isotope, deuterium, brings about a decrease of acidic strength and a slight increase in vapor pressure. Both these changes are attributed to a greater stability of 2-covalent deuterium, which results in an increase in the extent of association to form dimeric molecules.

Polydentate Chelate Rings. Organic molecules containing two coordination centers frequently give rise to di- and tricyclic systems of spirane type, as indicated in a number of structures previously cited (e.g., formulas III, IV, p. 1640). When three or more coordination centers are present, condensed chelate structures may be produced, and these have been designated as tri- and quadridentate systems. A tridentate structure, analogous to the condensed rings of naphthalene, is present in the metallic complexes of diethylene triamine (I), and a quadridentate system (II) in the complexes from bis-acetoacetonyl ethylenediamine.⁴⁰



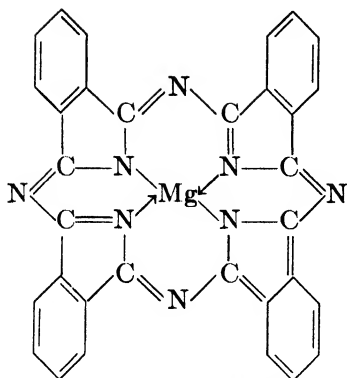
A particularly interesting quadridentate structure is produced from the phthalocyanines; these metallic complexes are obtained readily by the action of iron or magnesium oxide upon *o*-cyanobenzamide and derive their name from their deep blue color.⁴¹ There is a close structural resemblance between the phthalocyanines and the porphyrins, which form the basis of many important natural pigments (hemoglobin, chlorophyll) and have been shown to contain the "porphin" ring system.

³⁸ Sidgwick, "Ann. Repts. Chem. Soc. (London)," **30**, 115 (1933).

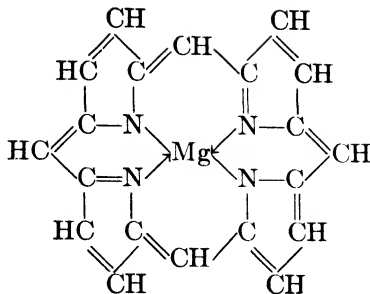
³⁹ Lewis and Schutz, *J. Am. Chem. Soc.*, **56**, 493, 1002 (1934).

⁴⁰ Morgan and Main Smith, *J. Chem. Soc.*, 912 (1926).

⁴¹ Linstead and collaborators, *ibid.*, 1016, 1031, 1033 (1934); "Ann. Repts. Chem. Soc. (London)," **32**, 361 (1935).



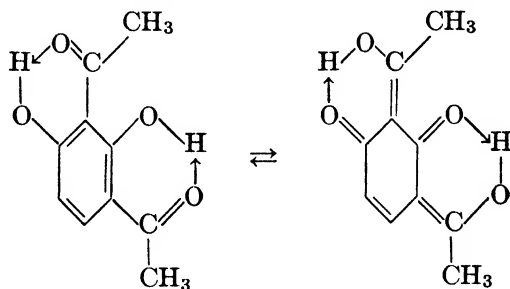
Magnesium derivative of phthalocyanine



Magnesium derivative of porphin type

The synthetic phthalocyanines bear a close analogy to the natural porphin structure, but are different in two features: each of the four pyrrole units of the phthalocyanines bears a condensed benzene ring in the 3,4-positions, and the units are connected by nitrogen instead of CH groups. These differences do not influence the molecular configuration or stability very seriously, and there are strong resemblances between them. Both are stable to alkalis, less so to acids; both are highly colored and form metallic complexes of similar stability. Thus, the magnesium derivative of a porphin type (phytychlorin, phytyrhodin) or a phthalocyanine is intermediate in stability between the potassium salt, which is de-metalated in dilute alcohol, and the very stable copper derivative.

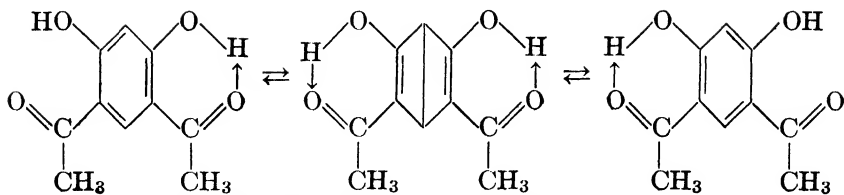
Orientation Effects of Chelation. A definite influence of the effect of conjugation in a chelate structure upon the mobility of the double bonds in an aromatic system has been demonstrated by Baker and his collaborators.⁴² Physical properties indicate that 2,4-diacetylresorcinol (I) is fully chelated and that this has an effect of fixing the



I. 2,4-Diacetylresorcinol (phenanthrene type)

m.p. 89° , b.p. $168^{\circ}/10\text{mm.}$; volatile with steam

⁴² Baker and collaborators, *J. Chem. Soc.*, 1684 (1934); 628 (1935); 274, 346 (1936).



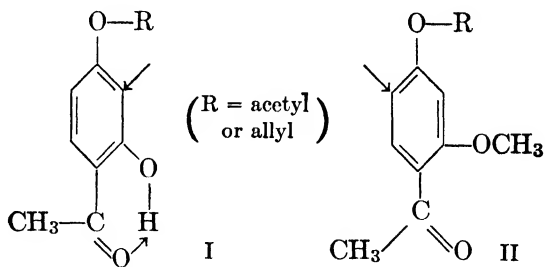
II. 4,6-Diacetylresorcinol (anthracene type)

m.p. 182°, b.p. 188°/10 mm.; non-volatile with steam

positions of the double bonds of the aromatic structure (p. 75). In the isomeric 4,6-diacetylresorcinol (II), the chelation appears to be less complete since the effects of the chelation upon the double bonds in the aromatic system would oppose each other, and effective participation of the aromatic system would require the production of a *para*-bridged, quinonoid structure. The two systems are analogous to phenanthrene and anthracene, respectively, and there should be little or no fixation of the aromatic double bonds in the 4,6-isomer.

On the basis of the chelation theory Baker predicted that in the Fries reaction 4-acetoxy-2-hydroxyacetophenone (I) should give 2,4-diacetylresorcinol rather than the 4,6-isomer. The reaction was found to give about 60 per cent of the predicted product and 40 per cent of the 4,6-isomer. This result indicates a marked orientation effect since the methyl ether of 4-acetoxy-2-hydroxyacetophenone (II), which cannot be chelated, gives almost entirely the 4,6-derivative.

Similar effects were observed in the rearrangement of 4-allyloxy-2-hydroxyacetophenone. The principal product was the 3-allyl derivative, but if the original compound was methylated before rearrangement, the allyl group entered the 5-position.



"Oriented" rearrangement

"Normal" rearrangement

The influence of chelation upon the alkaline cleavage of *N*-acylated benzoin oximes has been suggested by Blatt⁴³ to account for marked

⁴³ Blatt, Barnes, and Russell, *J. Am. Chem. Soc.*, **57**, 1330 (1935); **58**, 1900, 1903 (1936).

differences in the behavior of the α - and β -forms. The facile cleavage into benzonitrile and benzaldehyde occurs only with the α -(*anti*-CHOH)-forms, and the β -forms are merely deacylated by alkalis. Examination of isomeric *N*-acetylated *o*-hydroxybenzophenone oximes reveals a similar effect; only the *anti*-hydroxyaryl forms undergo smooth rearrangement to benzoxazoles and the *syn*-forms are simply deacylated.

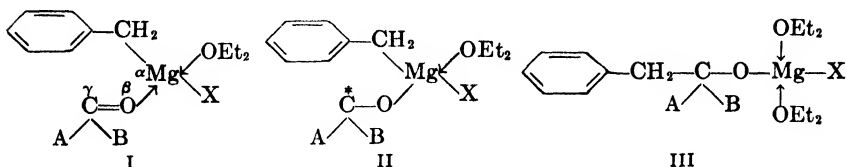
Chelation in Chemical Reactions. It has been stated that the powerful catalytic effects of certain metals and salts may be attributed to the formation of unstable coordination complexes, and in certain cases the observed course of reaction suggests that a transitory chelation takes place in the unstable complex (p. 1635). This hypothesis affords a new point of view for the interpretation and correlation of reactions that are not adequately elucidated by the conventional mechanisms. Specific applications of the hypothesis of transient chelation may be illustrated by a consideration of certain "abnormal" reactions of Grignard reagents (pp. 431 and 1651).

Studies of the behavior of benzylmagnesium chloride toward a variety of reactants has shown that certain carbonyl compounds (formaldehyde, ethyl formate, acid chlorides, and anhydrides) give rise to *o*-tolyl derivatives, but a number of others (carbon dioxide, ketones, and typical esters) produce only the expected benzyl compounds.⁴⁴ The experimental evidence shows clearly that the reactant itself plays an important role; the assumption of dynamic isomerism between a normal and *o*-quinonoid form of the Grignard reagent, or rearrangement of a *free* benzyl anion in the course of reaction, does not give a satisfactory account of the observed results. There is also definite evidence against either of these assumptions.

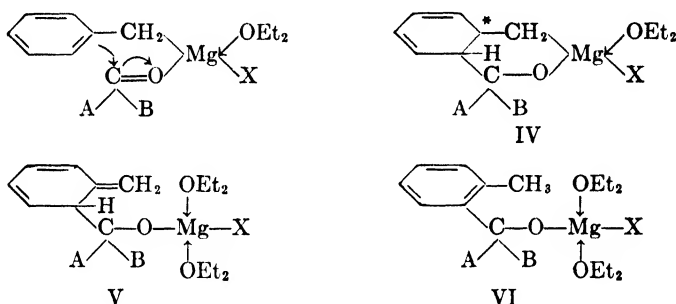
On the basis of the chelation theory,²⁹ the normal and abnormal reactions are regarded as two possible courses of transposition within the initial coordination complex which is formed as the first step in all Grignard reactions. A carbonyl compound $A-CO-B$ combines with the Grignard reagent, by means of an unshared electron pair of the carbonyl oxygen, to give the initial complex I. The coordination process $C=O \rightarrow Mg$ tends to favor electron withdrawal by the benzyl group in the link $Mg-CH_2C_6H_5$, and to promote in the carbonyl group an electromeric displacement $C \overset{\curvearrowright}{=}\overset{\curvearrowleft}{O}$ which would leave the carbon atom with a sextet of electrons (marked by an asterisk, formula II). The normal Grignard reaction occurs by a direct α,γ -shift of the benzyl group with its binding electrons, and without internal rearrangement, to the deficient carbon of the carbonyl system. The octet of the magne-

⁴⁴ Gilman and Kirby, *ibid.*, **54**, 345 (1932); Austin and Johnson, *ibid.*, **54**, 647 (1932)

sium atom is completed by the usual coördination with ether, and the stable normal product results (III).



The abnormal reaction arises as a result of the ability of the allylic system in the Grignard reagent to forestall the normal reaction by furnishing the mobile electron pair of the *ortho*-double bond to the deficient carbon atom. The ephemeral chelate ring is broken by rupture of the magnesium-carbon linkage (and coördination of the magnesium with ether) to give the product V

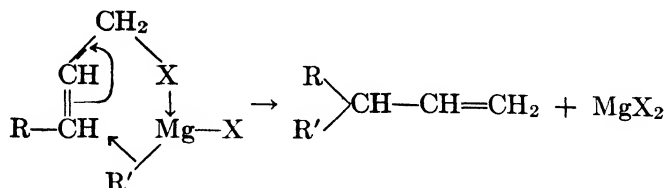


In aliphatic allylic systems the reaction may go no further, but with the aryl compounds a proton shifts to the side chain and completes the conversion of the benzyl group into an *o*-tolyl group. The tendency of a series of carbonyl compounds to bring about the abnormal reaction is clearly influenced by the nature of the atoms A and B, and parallels the reactivity in typical carbonyl reactions: the most active carbonyl compounds favor the abnormal reaction.

The allylic rearrangements observed by Prévost⁴⁵ in the reaction of $R-CH=CH-CH_2Br$ and $R'-MgX$, to give $R-CHR'-CH=CH_2$ and the normal product $R-CH=CH-CH_2-R'$, may be explained by a mechanism analogous to that given above. In these cases the allylic group of the reactant is responsible for the abnormal reaction; furthermore, the process is arrested at the stage corresponding to structure V. Obviously the double bond is less mobile here than in an *o*-quinonoid

⁴⁵ Prévost, *Ann. chim.*, [10] **10**, 121 (1928); Prévost and Daujat, *Bull. soc. chim.*, [4] **47**, 588 (1930); see, also, Carothers and Berchet, *J. Am. Chem. Soc.*, **55**, 2813 (1933).

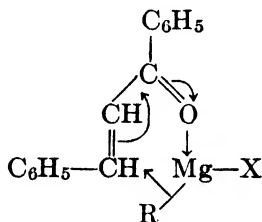
structure. Other Grignard reactions that appear to involve an ephemeral cyclization are the 1,4-addition reactions of α,β -unsaturated ketones and esters,⁴⁶ and *o*-phenylations of benzophenone anil⁴⁷ and highly



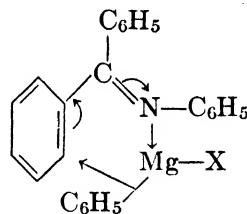
Abnormal reaction of Grignard reagents and allylic halides

substituted α,β -unsaturated ketones⁴⁸ by forced reaction with phenylmagnesium bromide.

In the initial complex derived from an α,β -unsaturated carbonyl compound (I) two courses of reaction are possible: (i) the "normal" α,γ -shift of the Grignard group R to give 1,2-addition; (ii) shift of the R group (ephemeral cyclization) to the β -carbon of the carbonyl system,



I. 1,4-Addition



II. *ortho*-Phenylation

in concurrence with an electron drift toward the carbonyl group. In this type of reaction it is observed that the most reactive carbonyl systems (aldehydes) (p. 1690) favor 1,2-addition, and less reactive types ($-\text{CO}-\text{C}_6\text{H}_5$, $-\text{CO}-\text{OR}$) 1,4-addition. *o*-Phenylation (II) of an aryl group attached to the carbonyl system occurs only when steric factors interfere with the 1,2- or 1,4-addition.

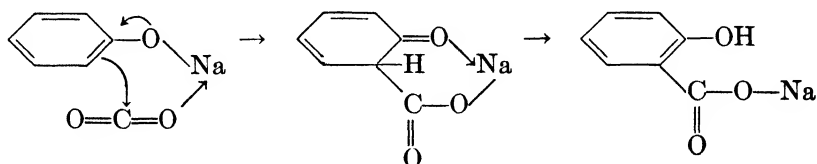
The chelation hypothesis is of rather general application and is not restricted to abnormal reactions. The O- and C-alkylation of metallic enolates represent alternative courses of reaction that are analogous to the examples given above: α,γ -shift leads to O-ethers, and the cyclic mechanism to C-alkylation. The Kolbe synthesis and the Reimer-Tiemann reaction may also be formulated by a cyclic mechanism.²⁹

⁴⁶ Kohler, *Am. Chem. J.*, **38**, 511 (1907), and later papers; see also, pp. 422 and 581.

⁴⁷ Gilman, Kirby, and Kinney, *J. Am. Chem. Soc.*, **51**, 2252 (1929).

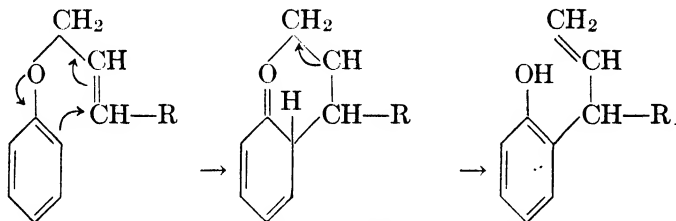
⁴⁸ Kohler and Nygaard, *ibid.*, **52**, 4128 (1930).

It may be pointed out, however, that the mere circumstance that a plausible cyclic mechanism can be written for a reaction does not indicate *per se* that the reaction can take place only by a cyclization process. Thus, the rearrangement (p. 747) of allyl phenyl ethers⁴⁹ may occur

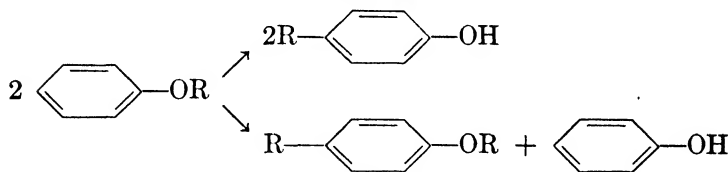


Chelate formulation of the Kolbe synthesis

by an intramolecular (cyclization) process, but it may also take place by an intermolecular alkylation.⁵⁰ The chelation hypothesis of transient cyclization has this advantage: it offers a definite basis for predicting or correlating the influence of structural factors, or variations in experimental conditions (nature of the medium, etc.), upon the course of a given reaction. In a number of instances the observed effects are in good agreement with those anticipated from theoretical considerations.



Intramolecular rearrangement (cyclic)



Intermolecular alkylation

In the rearrangement of benzyl and α -furfuryl phenyl ethers the participation of the allylic double bond in the cyclization (intramolecular) mechanism is diminished by virtue of conjugation in the ring system of benzene or furan, and consequently the intermolecular mechanism is favored. In both of these cases the "rearrangement" is observed to occur preferentially in the *para*-position, and a certain amount of the

⁴⁹ Ingold, "Ann. Repts. Chem. Soc. (London)," **23**, 134 (1926).

⁵⁰ Smith, *J. Am. Chem. Soc.*, **56**, 717 (1934).

para-alkylated ether and free phenol can be isolated from the reaction mixture.

ELECTRONIC CHARACTERISTICS OF TYPICAL BONDS

Unsymmetrical Single Bonds. If the single links of carbon with other elements are regarded from the standpoint of the electronic configuration of the valence shell of the hetero atom in the compound, they fall into three broad classes: (1) links in which the hetero atom has an incomplete valence shell and would require one, two, or three additional electron pairs to form an octet; (2) those in which the hetero atom has a completely shared octet (doublet, in the case of the C—H link) but is capable of increasing its covalence and acquiring additional shared electron pairs by coördination; (3) those in which the hetero atom has an octet containing one, two, or three unshared electron pairs. The first category embraces atoms in Groups I, II, and III of the periodic table; the second, hetero atoms in the higher periods of Group IV; and the third includes hetero atoms in Groups V, VI, and VII. The C—H bond and unsymmetrically substituted C—C bonds may be regarded as special cases in the second class, but they merit individual consideration. Typical examples illustrating the general classification are shown below.

TABLE XII

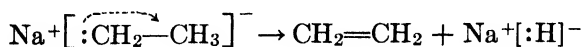
Classification of Links between Carbon and Hetero Atoms

Class 1	Class 2	Class 3
Li—C ₂ H ₅	(C ₂ H ₅) ₃ Si—C ₂ H ₅	(C ₂ H ₅) ₂ N—C ₂ H ₅
C ₂ H ₅ —Be—C ₂ H ₅	(C ₂ H ₅) ₃ Ge—C ₂ H ₅	C ₂ H ₅ —O—C ₂ H ₅
C ₂ H ₅ —Zn—C ₂ H ₅	(C ₂ H ₅) ₃ Sn—C ₂ H ₅	F—C ₂ H ₅
(C ₂ H ₅) ₂ B—C ₂ H ₅	(C ₂ H ₅) ₃ Pb—C ₂ H ₅	I—C ₂ H ₅
<i>etc.</i>	<i>etc.</i>	<i>etc.</i>

Class 1 (Groups I, II, III). In links of the first class, owing to the lower effective nuclear charge of the hetero atom relative to carbon, a permanent inductive displacement (I_s) will occur toward the carbon atom. These hetero atoms will be considered to exert a negative inductive effect upon the carbon atom, and the latter a positive effect on the hetero atom. When these links are ruptured in the course of reaction the inductive effects will facilitate the separation of the organic group *with* the binding pair of electrons, but the mechanism of reaction involves a consideration of the contribution of coördination processes and of polarizability effects.

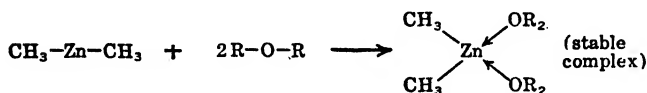
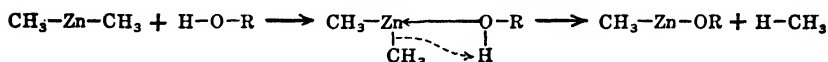
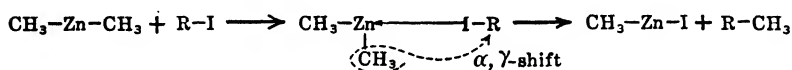
In ethylsodium, and the alkali alkyls in general (p. 440) the normal state of the molecule is essentially electrovalent. The decomposition

of ethylsodium, at temperatures below 100° , into sodium hydride and ethylene may be attributed to the instability of the *free* ethyl anion.⁵¹



The alkali alkyls are valuable diagnostic reagents for proton mobility,⁵² and their high proton affinity, or nucleophilic activity in a broader sense, is likewise to be associated with the intervention of alkyl anions. The separation of free hydrocarbon anions cannot be doubted in the case of the α -phenylated alkyls; benzylsodium and its analogs are highly colored substances, and their solutions in appropriate media are electrical conductors.

The reactions of the alkali metal compounds are not usually typical of the behavior of the links of other hetero atoms in this class, and in general the assumption of *free* hydrocarbon anions as intermediates is dubious and unwarranted by the experimental facts. Reactions of the *typical* links appear to involve two steps: the formation of a primary unstable coördination complex in which the hetero atom acts as an acceptor and a subsequent migration (usually an α,γ -shift) of the nascent hydrocarbon anion *within* the complex. There can be little doubt that the initial step in the typical reactions of the Grignard reagents is a coördination process in which the magnesium acts as an acceptor and the reactant furnishes an active donor center; much experimental evidence supports the view that nearly all the hetero atoms in this class act in a similar way.



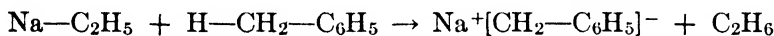
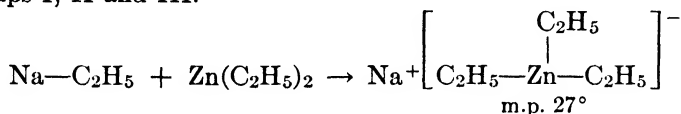
In the presence of donor reactants the alkali metal compounds may behave in the same fashion, and in solutions in relatively non-reactive donor solvents (aliphatic ethers and tertiary amines) they may exist as unionized solvated complexes in equilibrium with hydrocarbon anions and solvated cations.

The distinctive reactions of the alkali alkyls appear to be associated

⁵¹ Carothers and Coffmann, *ibid.*, **51**, 568 (1929).

⁵² Conant and Wheland, *ibid.*, **54**, 1212 (1932).

with a facile inductomeric polarization, as a result of which they are capable of transferring an alkyl anion to an acceptor reactant without the intervention of a donor center of the reactant or of a donor solvent. For instance, ethylcesium, -rubidium and -sodium are capable of converting diethylzinc into the triethylzinc anion, from which the volatile diethylzinc (b.p. 118°) cannot be removed by heating. The alkali alkyls react also with the H—C link of benzene, the aliphatic H—C link of toluene, and with ethylenic double bonds. None of these reactions appears to occur with Grignard reagents⁵³ or with other metal alkyls of Groups I, II and III.



It is of interest to compare the behavior of R—Na, R—Mg—X (or R₂Mg), and R₂AlX (or R₃Al) toward the same reactant. With acetone, the first reacts mainly as an enolizing agent, the Grignard reagent gives an addition product that yields a tertiary alcohol upon hydrolysis, and the organoaluminum compound produces mainly mesityl oxide and higher condensation products. With certain other reactants the organoalkali compounds and the corresponding Grignard reagents yield identical products and differ merely in their rates of reaction (p. 440).⁵³ Explanations of the observed differences in behavior of organometallic compounds can be inferred from a consideration of relative polarization and polarizability effects within the reacting molecules (internal factors) and the influence of the environment (external factors).^{*} The position of equilibrium and the mobility (rate of change) within a system are independent variables; the nature of the products of a reaction will depend upon the relative importance of the contributions of the two factors and their influence upon competitive reactions (p. 804).

In links of the first class the magnitude of the inductive polarization effects ($-I_s$) and the tolerance of the hetero atom for a positive charge (polarizability) change in the same way, and vary in a regular manner with the position of the hetero atom in the periodic table. Both quantities diminish as the atom moves to the right in the first two periods

⁵³ Gilman and Kirby, *ibid.*, **55**, 1265 (1933).

^{*} Internal factors involve the influence of substituents and their mutual interaction, atomic dimensions and steric effects; external factors take into account the electronic characteristics of the medium and catalysts (if any), and the effect of temperature, concentration, photochemical excitation, etc.

(Li > Be > B and Na > Mg > Al), and increase in passing from the first to the second period within each group. Polarization and polarizability increase in passing into the A-subgroup toward the elements of higher atomic number (Li < Na < K < Rb) but decrease in going into the B-subgroups (Mg > Zn > Cd > Hg). The factors associated with the direction of these changes are the effective nuclear charge and the size of the atom (effective atomic and ionic radii) * and its electronic configuration. The marked differences between elements in the A and B subdivisions of Groups I, II, and III involve the relatively smaller effective radii of the elements of the B-subgroups (p. 1657) and the fact that their atomic kernels do not possess an electronic configuration of inert gas type.

The relative acceptor activity of the hetero atoms in Groups I, II, and III may be approached in a roughly qualitative way from the aspects of residual charges in the link, the effective nuclear charges and atomic radii, and the nature of the electronic configurations. The tendency of an atom to ionize and its ability to act as an acceptor (or a donor) are independent properties and are complementary in nature. Both may be seen to proceed from the operation of two fundamental principles: the tendency of an atom to approach the stable electronic configuration of an inert gas, and to achieve a maximum electronic neutralization of its nuclear charge (minimum residual atomic charge).

As the permanent polarization in a covalent link becomes larger the dynamic increment (activation) required for the withdrawal of the binding electrons by the incipient anion becomes smaller, but at the same time there is an increase in the electrostatic attraction between the atoms and an increase in their tendency to form additional links by coördination (subject to the maximum covalence rule, p. 1603). From considerations based upon the optical properties of inorganic salts, Fajans⁵⁴ has shown that ions are not rigid structures, and has related the process of ionization to the mutual deforming power of the potential ions (polarization effects) and their susceptibility to deformation (deformability, polarizability).

In inorganic salts the deformation is essentially that of the anion under the influence of cation as a deforming agent; but in the case of a small anion and a large cation (as in potassium fluoride) the effect of the anion may predominate. Fajans observed that the amount of deforma-

* The term effective radius is used to indicate the contribution which the atom may be regarded as making to the distance between the two atomic nuclei in the link. The effective radius of an atom increases in passing from an electrically neutral state to that of an anion, and diminishes in becoming a cation.

⁵⁴ Fajans, "Radioelements and Isotopes: Chemical Forces and Optical Properties of Substances," McGraw-Hill Co., New York (1931).

tion in inorganic salts is greater: (i) the larger the ionic charge; (ii) the smaller the cation; (iii) the larger the anion; (iv) for cations that do not possess an inert gas configuration. These generalizations are in agreement with those predicated from theoretical considerations, and the known behavior of a large number of inorganic compounds supports the inference that the tendency of a covalent molecule to ionize is restricted by the amount of deformation of the potential ions.^{4, 5} The approximate radii of some of the typical univalent ions, calculated by Pauling,^{5, 5} are shown in Table XIII.

TABLE XIII
APPROXIMATE UNIVALENT CRYSTAL RADII OF IONS (PAULING)
(in Ångström units)

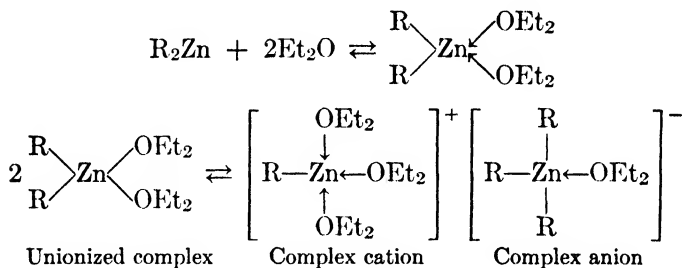
<i>Univalent Cations</i>						
Li	Be	B	C			
(0.60)	(0.44)	(0.35)	(0.29)			
Na	Mg	Al	Si			
(0.95)	(0.82)	(0.72)	(0.65)			
A-Subgroups			B-Subgroups			
K	Ca	Cu	Zn	Ga	Ge
(1.33)	(1.18)		(0.96)	(0.88)	(0.81)	(0.76)
Rb	Sr	Ag	Cd	In	Sn
(1.48)	(1.32)		(1.26)	(1.14)	(1.04)	(0.96)
Cs	Ba	Au	Hg	Tl	Pb
(1.69)	(1.53)		(1.37)	(1.25)	(1.15)	(1.06)
<i>Univalent Anions</i>						
C	N	O	F	H		
(4.14)	(2.47)	(1.76)	(1.36)	(2.08)		
Si	P	S	Cl			
(3.84)	(2.79)	(2.19)	(1.81)			
Ge	As	Se	Br			
(3.71)	(2.85)	(2.32)	(1.95)			
Sn	Sb	Te	I			
(3.70)	(2.95)	(2.50)	(2.16)			

Ionization of weak electrolytes, or the rupture of a covalent bond in the course of reaction, usually involves the intervention of coördination processes, as a result of which the amount of deformation of one or both of the incipient ions is reduced. Coördination of a cationic (electrophilic) center with a donor will reduce its deforming power owing

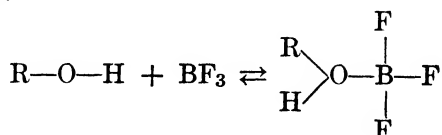
⁵⁵ Pauling, *J. Am. Chem. Soc.*, **49**, 771 (1927).

to the production of a more stable valence shell and to the dissemination of the residual positive charge; coördination of an anion with an acceptor center will reduce its deforming power and its deformability, since the residual negative charge and electron mobility are thereby diminished.

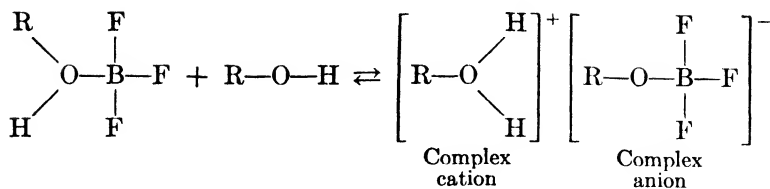
Diethylzinc is not appreciably ionized in the pure state and is a very poor conductor, but its conductivity is increased greatly by the addition of anhydrous ether, which is also a poor conductor. The effect of the ether can be attributed to the formation of etherates in which the deforming power of the cation $(R-Zn)^+$ is reduced. The alkyl anion, by coördination with unionized diethylzinc, can be converted also into a complex anion $[R_3Zn]^-$, of greatly diminished deformability. These relations are shown in the equilibria given below; a similar situation occurs in the usual ethereal solutions of Grignard reagents, giving rise to solvated molecules of R_2Mg , $RMgX$ and MgX_2 , and to solvated ions such as $[RMg + 3Et_2O]^+$, $[R_3Mg + Et_2O]^-$, etc.



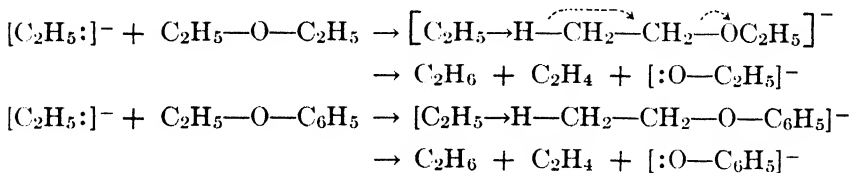
The introduction of an acceptor molecule such as BF_3 or $Al(OEt)_3$ can increase the ionization of an extremely weak acid such as ethyl alcohol.^{27, 56} With reference to the ionization of acids Latimer and Rodebush¹³ have made this statement: "It is doubtful if the hydrogen nucleus ever gets very far away from one or more electrons . . . the ionization of acids, or extreme polarity of any compounds involving hydrogen, must be interpreted as due to the transfer of a hydrogen nucleus from one molecule to another, thus forming a complex ion." The action of BF_3 and similar acceptor molecules is shown in the following equations:



⁵⁶ Nieuwland and others, *ibid.*, **52**, 1018, 2892 (1930); **53**, 3835 (1931); **54**, 2017 (1932).



The ionization of acids and bases, and reversible chemical reactions in general, may be considered from the same standpoint. The completion of a chemical reaction merely involves the occurrence of an ionic displacement that is not reversible under the influence and conditions of the environment. This may be illustrated by the behavior of ethylsodium toward ether. The ether facilitates ionization, as it does with diethylzinc, but the relative reluctance of sodium to take part in forming a complex anion leads to an attack of the ether by the alkyl anions. The resulting anionic complex is unstable and decomposes irreversibly, with the elimination of ethane and ethylene, to form the more stable



ethoxide anion. Ethylsodium attacks phenetole in a similar way and gives finally sodium phenoxide.

In covalent links of the hetero atoms in Groups I, II, and III, permanent acceptor activity will be limited primarily to the hetero atom itself but temporary acceptor activity may be conferred upon hydrogen in H—C links of an attached group by means of dynamic effects ($+I_d$) and may be effective in the course of chemical reaction. These effects will be expected to occur particularly with hetero atoms having low polarizability and furnishing a potential cation of high deforming power, such as 3-covalent boron.* In saturated alkyl derivatives of boron the $+I_d$ effect is opposite in direction from the permanent polarization but might become important when the attachment of a donor center to the boron atom itself is impeded for steric reasons.

In the event that the atom attached to boron bears an unshared electron pair or a multiple covalent bond an electromeric shift can occur

* The effective radius of the univalent boron cation has been estimated by Pauling⁵⁵ to be 0.35 Å; its deforming power should exceed that of any univalent cation except carbon (0.29 Å), nitrogen (0.25 Å), oxygen (0.22 Å), fluorine (0.19 Å), or hydrogen.

toward the boron atom, so that its electron deficiency will be diminished: $R_2B \overset{\curvearrowright}{O}-C_2H_5$ and $R_2B \overset{\curvearrowright}{C} \overset{\alpha}{=}\overset{\beta}{C}-R$. If a vinyl group is present, the $+E$ effect of boron will result in a transfer of the electron deficit to the *beta*-carbon atom (see below, Hetero-enoid systems). If an aryl group is present, the effect in the ring is similar to that of a nitro or carboxyl group and will cause a diminution in the ease of substitution and favor *meta* orientation.⁵⁷



The electromeric shift of an unshared electron pair from the adjacent atom toward boron should diminish the acceptor activity of 3-covalent boron; the fact that BF_3 is a more powerful acceptor than $B(OC_2H_5)_3$ may be anticipated from the ability of the alkoxy group to permit a greater electronic displacement ($-E$ toward boron) than fluorine does⁵⁸ $R-\overset{\curvearrowright}{O}-B > F-B$.

The stability of coordination complexes and the relative ease of ionic displacement within them are influenced by a variety of factors: the relative sizes of the donor and acceptor atoms, the spatial arrangement about the coordination center, the intervention of chelation and of resonance effects (tautomeric degeneracy). The ability of an atom to act as an acceptor is affected by the electronic and steric characteristics of the attached groups, and the number of additional covalent links is limited by the maximum covalence rule. However, an atom directly combined to one or more hydrocarbon radicals rarely forms a stable complex in which its valence shell is expanded beyond an octet. A few exceptions have been cited previously (p. 1611), e.g., R_3PCl_2 , $RAsCl_4$, $[CH_3-TeI_4]^-$, etc.; all of these have two or more halogen atoms attached simultaneously with the organic groups, and even in this favorable situation the systems show a strong tendency to revert to an octet.

Some of the remarkable differences in behavior which are found in comparing elements of the first horizontal period with those in the second and higher periods of the same group can be explained on the basis of the ability of the larger atoms to exceed a covalence of four as a transient intermediate step in their reactions (p. 1611). Certain other divergences have been accounted for by the hypothesis that an

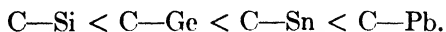
⁵⁷ Seaman and Johnson, *ibid.*, **53**, 711 (1931).

⁵⁸ Yabroff, Branch, and Almquist, *ibid.*, **55**, 2935 (1933).

atom which is capable of becoming 6-covalent can assume, although it does not usually do so, a plane space-distribution of its valences (angle 90°) in the 4-covalent state. Consequently such atoms would take part more readily in forming, and would give more stable, four-membered rings than the corresponding atoms of the first horizontal period. This may be the reason why boron, which is the only element in Group III incapable of becoming 6-covalent, is also the only element in that group to form trihalides that are not polymerized.⁴ A four-membered chelate ring structure for aluminum chloride (Al_2Cl_6) would be essentially strainless on this assumption (p. 1645) but a similar ring for boron trichloride would have a large strain if boron is restricted to the tetrahedral configuration (angle 109°).

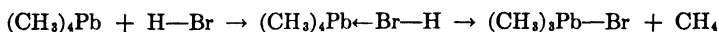
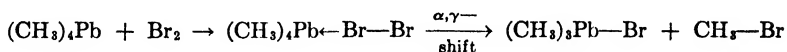
An explanation of the high catalytic activity of the chloride and alkoxides of aluminum, and of the almost complete absence of similar properties in the corresponding boron compounds, may be sought along the lines outlined above. Owing to the limited knowledge of the effects of coördination upon the donor and acceptor centers, and the recognized complexity of the factors governing the behavior of coördination complexes, it is to be expected that detailed predications cannot be made upon a firm basis at the present time. Nevertheless the coördination hypothesis offers the attractive possibility of interpreting and correlating a large number of important phenomena in a more precise manner and of regarding them as manifestations of the same fundamental principles.

Class 2 (Group IV). In the organic derivatives of silicon and the elements of the B-subgroup of Group IV (germanium, tin, and lead), the permanent polarization and the polarizability effects will be in the same direction as that in the compounds of elements in Groups I, II, and III. Owing to the larger effective nuclear charges of the hetero atoms in Group IV, and the presence of an octet of electrons in the valence shell of the hetero atom, the magnitude of the $-I_s$ effect and the polarizability will be smaller than that for the corresponding elements of the earlier groups: $\text{C}-\text{Si} < \text{C}-\text{Al} < \text{C}-\text{Mg} < \text{C}-\text{Na}$; $\text{C}-\text{Ge} < \text{C}-\text{Zn}$; $\text{C}-\text{Pb} < \text{C}-\text{Hg}$, etc. Within the fourth group the effects will increase toward the larger atoms:

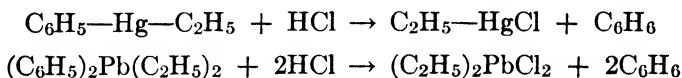


In these links the tendency to yield alkyl anions will be relatively small and their reactivity will depend largely upon the ability of the hetero atom to act as an acceptor by a temporary expansion of its valence shell beyond an octet. The stepwise dealkylation of the

tetraalkyl derivatives by halogens, and the reactivity of these compounds in general, may be explained readily on this assumption.



The observation that the cleavage of analogous unsymmetrical derivatives of mercury and lead by means of hydrogen chloride^{59, 60} gives the same products confirms the notion that the mechanism of reaction is essentially the same in both cases (p. 434).



When electromeric effects can intervene these heteroatoms may exert a $+T$ effect which will oppose the I_s effect, and the situation is similar to that outlined in the case of boron. Consequently, these hetero atoms may be expected to exert a *meta* directive influence (as well as *ortho-para*) in aromatic substitution reactions. The nitration of phenylgermanium trichloride ($\text{C}_6\text{H}_5-\text{GeCl}_3$) with fuming nitric acid at low temperatures has been found to give 58% of *meta* and 42% of *para* substitution;⁶¹ under similar conditions phenylboric acid, $\text{C}_6\text{H}_5-\text{B}(\text{OH})_2$, gives 85% of *meta* and 15% of *ortho* substitution.⁵⁷ Using acetic anhydride and a minimum excess of nitric acid, phenylboric acid gives almost exclusively *ortho* substitution. This reversal of orientation may be attributed to an opposing, secondary effect in an addition compound of phenylboric acid with acetic anhydride.⁵⁸ When the weak $+T$ effects of boron and germanium are reinforced by an appropriately situated methyl group, as in the *p*-tolyl derivatives, nitration occurs entirely in the *meta* position (with reference to Ge or B).

The sharp difference in the behavior of the C—C link and the links C—Si, C—Sn, and C—Pb is due to the inability of carbon to expand its valence shell to a decet. The attack of a donor molecule *must* occur through an H—C link of a substituent group. The action of chlorine on neopentane results in a substitution process in one or more of the methyl groups (dehydrogenation) and not in a replacement of the alkyl group (dealkylation) such as occurs with tetramethylsilicane.

⁵⁹ Kharasch and Flenner, *ibid.*, **54**, 674 (1932).

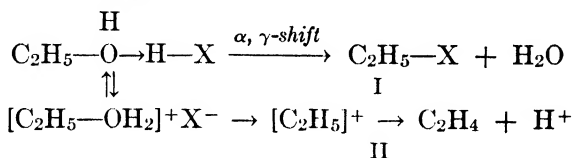
⁶⁰ Gilman, Towne, and Jones, *Rec. trav. chim.*, **51**, 1054 (1932); *J. Am. Chem. Soc.*, **55**, 4689 (1933).

⁶¹ Shelton, Washington Meeting, Am. Chem. Soc. (1933).

The tendency of these hydroxylic compounds to form dimers of the type $\text{H}-\text{OH}\leftarrow\text{OH}_2$ by intermolecular association falls off in the same direction and must be attributed largely to the diminishing acceptor activity of the active hydrogen.

The capacity of the oxygen atom to act as a donor will be enhanced as electron-release by the R group increases since this augments the mobility of the unshared electrons in its valence shell. Consequently the tendency of the alcohols to form oxonium salts, or complex cations in general, should increase in the order: primary < secondary < tertiary alcohols. The oxonium complexes derived from the simple alcohols are highly active systems and their behavior involves a consideration of alternative courses of reaction (see pp. 1635 and 1636 for example): formation of olefins, alkylation reactions, simple ionization, etc.

Coördination of the oxygen atom with a proton (or other acceptor) will result in a strong tendency to withdraw the electron pair of the link $\text{C}-\overset{\curvearrowright}{\text{O}}\text{H}_2$. The mobility in the oxonium complex will allow a suitable approach of the donor center and the potential alkyl cation; reaction I is the result of an α, γ -shift within the complex. Reaction II requires the intervention of polarizability effects, which determine the tendency of an alkyl residue to separate from the complex as a *free* cation. The alkyl cations will be extremely unstable owing to the electron deficit (open sextet) of the *alpha*-carbon atom, and will revert to a more stable configuration by ejecting a proton and forming an olefin (or a cyclic structure). The effect of substituents upon the course of rearrangements occurring in the dehydration of alcohols, and in many



similar processes, has been treated with remarkable success by Whitmore⁶² from the standpoint of the electronic configurations of the potential alkyl cations (p. 786).

Interpretation of the behavior of organic systems containing an aryl group directly attached to a reactive center ($\text{C}_6\text{H}_5-\text{X}$), or separated from it by an aliphatic system ($\text{C}_6\text{H}_5-\text{CH}_2-\text{X}$, $(\text{C}_6\text{H}_5)_2\text{CH}-\text{X}$, $\text{C}_6\text{H}_5-\text{CH}=\text{CH}-\text{X}$, etc.), involves a consideration of electromeric effects of aromatic groups and their interaction with other effects in the system and in the reagent. Experimental evidence supports the

⁶² Whitmore and collaborators, *J. Am. Chem. Soc.*, **54**, 3274, 3435, 3448 (1932).

view that aryl groups can exert dynamic effects of either sign and are capable of activating electron-attraction or electron-release, depending upon the nature of the reaction.

The ability of aryl groups to confer upon the attached atom an increased tolerance for an electrical charge of *either* sign is intimately associated with certain qualities represented by the term "aromaticity." The enhanced stability of the phenoxide and benzyl anions relative to their aliphatic analogs, and that of triarylmethyl cations relative to all other hydrocarbon cations, illustrate the intervention of dynamic effects of opposite sign. The dual polarizability effects may be attributed to the ability of the aromatic nucleus, by means of electromeric displacements in directions determined by the sign of the charge, to bring about a distribution of this charge primarily at the *para* and the *ortho* positions, and thence, by secondary inductive displacements, also to the *meta* positions, so that the residual atomic charge will ultimately be distributed over the entire system.⁷ Owing to the large number of possible positions for a residual charge the stability of the ions is enhanced. Influences of this kind (resonance effects) are significant in a number of organic compounds containing multiple bonds; the distinctive feature of aromatic systems (p. 1699) is their ability to compensate an electronic deficit or an electronic excess with nearly equal facility.

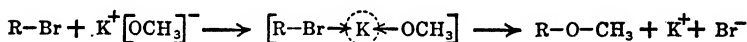
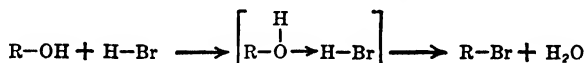
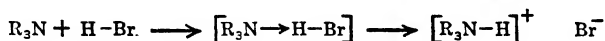
Class 3 (Groups V, VI, VII). The single links encountered most frequently in organic reactions are those of carbon to nitrogen, oxygen, and the halogens. The distinctive feature of the electronic configurations of these links is the presence of one or more unshared electron pairs in the valence shell of the hetero atom.

Owing to the higher effective nuclear charges of these hetero atoms relative to carbon, a permanent inductive displacement toward the hetero atom will take place in the typical single bonds ($+I_e$ effect with reference to carbon). The magnitude of the permanent polarization, and the deforming power of the hetero atom as a potential anion, change in a regular manner with the position of the hetero atom in the periodic table. These characteristics increase as the effective nuclear charge of the hetero atom becomes larger, i.e., as the atom moves to the right within each period— $N < O < F$, $P < S < Cl$, etc. The diminution of effective nuclear charge with increasing atomic radius causes the inductive effects to decrease within each group, in passing to the higher periods— $F > Cl > Br > I$, $N > P > As > Sb > Bi$, etc.

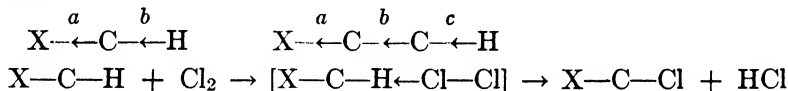
The deformability of the hetero atoms and the tendency of their unshared electron pairs to engage in electromeric effects (or donor activity) are properties related to the mobility of the electrons in the valence shell. These characteristics have an inverse relationship to the polariza-

tion effects of the hetero atoms and increase in the opposite direction. The mobility of unshared electron pairs decreases in going from left to right within the periods— $N > O > F$; $P > S > Cl$; etc. Within each group the electron mobility increases in passing into the higher periods— $I > -Br > -Cl > -F$; $-Sb > -As > -P > -N$; etc.

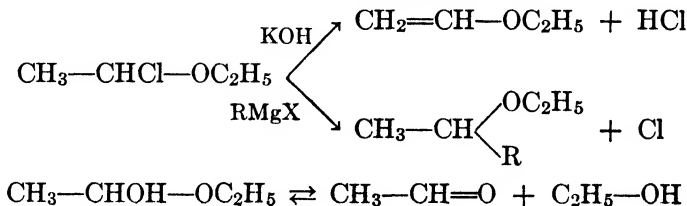
In saturated aliphatic systems donor activity is restricted to the hetero atom itself, but in unsaturated systems may be transmitted through an intervening chain (see hetero-enoid systems). Many of the typical reactions of the hetero atoms in Groups V, VI, and VII occur through coordination with an acceptor center of the reagent; a few examples are indicated briefly in the following equations:



The permanent polarization effects of these hetero atoms will tend to create an active center of low electron density (acceptor activity) in the system. The introduction of chlorine or ethoxyl in a hydrocarbon chain increases the electron deficit of the carbon to which it is linked (relative to $H-C$). This deficit is overcome in part by secondary inductive displacements in adjacent links, and by a similar mechanism is transmitted with successive diminutions to more distant atoms in the system.



As a result of the inductive effects of chlorine and oxygen, alkyl halides and ethers will react with donor reagents more readily than the parent hydrocarbons, and will undergo direct substitution preferentially on the alpha carbon atom. The presence of two hetero atoms in close proximity in an organic system will lead to an enhanced reactivity of the system; the result of a reaction in these systems will involve the intervention of polarizability effects, steric factors, etc.



The inductive effects of halogens and hydroxyl and alkoxy groups result in an increase in the strength of the acid when these substituents are introduced into an aliphatic system containing the carboxyl group. By means of a logarithmic function based upon the ionization constants of a series of substituted acids, Derick⁶³ developed certain generalizations concerning the influence of the substituent. If the inductive effect of a halogen atom in the alpha position is taken as unity, its influence in the beta, gamma, and delta positions in the isomeric acids is $\frac{1}{3}$, $\frac{1}{9}$, and $\frac{1}{27}$, respectively. More recently Hixon, Johns, and their collaborators⁶⁴ have shown that the polar properties of an extended series of organic compounds including $R-OH$, $R-CO_2H$, $R-CH_2-CO_2H$, $R-NH_2$, $R-HgX$, and others, can be expressed as an exponential function of an arbitrary number representing the "electron-sharing ability" of R (provided R does not contain polar groups).

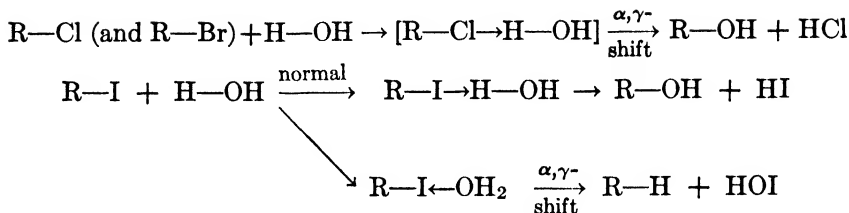
In the saturated aliphatic derivatives of nitrogen, oxygen, and fluorine, the participation of the hetero atom in coordination processes is limited to donor activity since their valence shells are restricted to an octet. In appropriate unsaturated and aromatic structures the donor activity may be transmitted through a chain of covalent bonds (see Hetero-enoid systems). Atoms of the second and higher periods can expand their valence shells beyond an octet, although they rarely form stable organic compounds with an enlarged valence shell. The capacity of these atoms to expand their valence shells, and the instability of the resulting configuration, suggest that under suitable conditions the mechanism of their reactions may involve acceptor activity of the hetero atom. This premise may be used as the basis for an explanation for certain distinctive reactions of the atoms within a given periodic group (see pp. 1611-1613); this view is supported by a good deal of indirect evidence.

Owing to the fact that the permanent polarization effects of these hetero atoms enhance the acceptor activity of adjacent atoms, it seems probable that acceptor activity of the hetero atom itself is associated largely with polarizability factors. It is observed that the general tendency to expand the valence shell beyond an octet is more prominent in the larger atoms and follows the trend of polarizability effects. The greater capacity of iodine to behave as a relatively "positive" atom in its reactions affords an illustration of this trend.⁶⁵

⁶³ Derick, *ibid.*, **33**, 1162, 1181 (1911).

⁶⁴ Hixon, Johns, and collaborators, *ibid.*, **49**, 1786 (1927); **50**, 168 (1928); **53**, 4367 (1931); **54**, 3971 (1932); *J. Phys. Chem.*, **34**, 2218, 2226 (1930).

⁶⁵ Nicolet and collaborators, *J. Am. Chem. Soc.*, **43**, 2081 (1921); **49**, 1796, 1801, 1806 (1927).



One may anticipate that the influence of substituents upon the normal reactions will be reversed for "abnormal" reactions, and this appears to be true. The reactivity of *n*-propyl chloride in a typical metathetical reaction is much greater than that of isopropyl chloride, but in an "abnormal" reaction, such as the formation of alkyl chlorides from the iodides and mercuric chloride, isopropyl iodide reacts at least fifty times more rapidly than *n*-propyl iodide.⁶⁶

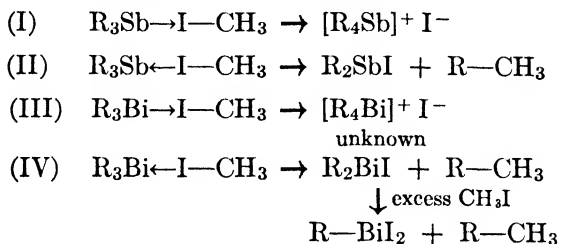
A comparison of certain reactions of the alkyl derivatives of arsenic, antimony, and bismuth with those of the corresponding elements of Group IV (tin, germanium, and lead) affords an interesting illustration of the influence of polarizability effects upon chemical behavior. The formation of salts of the type $[(\text{CH}_3)_4\text{Sb}]^+\text{Cl}^-$ may be taken as a characteristic reaction of the elements of Group V (B-subgroups); the removal of the alkyls by means of halogens is a typical reaction for the elements of Group IV, and earlier groups. The first reaction occurs readily when the alkyl derivatives of arsenic or antimony are treated with an active alkyl halide, but the alkyl bismuthines behave differently. Trimethylbismuthine reacts with methyl iodide upon warming but it yields ethane and methylbismuth di-iodide instead of a bismuthonium salt.⁶⁷ In this respect the bismuthine resembles alkyl derivatives of zinc and mercury rather than those of arsenic and antimony (p. 1669).

The anomalous behavior of the bismuthine suggests that the nature of the reaction is altered by a difference in the relative polarizability effects of antimony and bismuth with reference to iodine. The behavior of the reactants may be influenced by mesomeric effects and by electromeric effects within the complex. In the present instance it may be assumed that both reactions involve the coördination of iodine with antimony or bismuth but the initial complex can exist in two different "active" configurations (represented by I-IV). In one pair of these the iodine has an expanded valence shell (I and III), and in the other antimony and bismuth have expanded valence shells (II and IV); in

⁶⁶ Nicolet and Potts, *ibid.*, **50**, 212 (1928).

⁶⁷ Breed, *Ann.*, **82**, 106 (1852); Dünhaupt, *Ann.*, **92**, 371 (1854); Marquardt, *Ber.*, **20**, 1516 (1887); **21**, 2035 (1888).

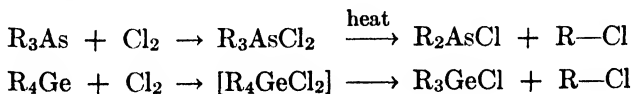
the formulas given below, the atom with an expanded shell is indicated by the direction of the arrow in the complex.



The course of the reaction appears in either instance to involve a stabilization of the more labile active form, leading to systems in which all the atoms have a normal valence shell. The active form of types I and III will be more labile in the stibine than the bismuthine, owing to the relatively smaller tolerance of antimony for a positive charge (smaller atomic radius). The effective nuclear charge of antimony is greater than that of bismuth, and the difference in effective nuclear charge between antimony and iodine is less than bismuth and iodine; consequently an α, β -shift of the potential alkyl anion occurs more readily from iodine to antimony than to bismuth.

The reaction of the bismuthine probably occurs through the active form IV, in which the high mobility of the expanded valence shell of bismuth facilitates separation of an alkyl anion; the larger atomic radius of bismuth allows a closer approach of the potential alkyl anion and the positively polarized alkyl group than the corresponding complex from the stibine. The view that the bismuthine reacts by way of the complex IV, rather than III, is consistent with the general resemblance of bismuth to the metallic elements in the earlier periodic groups; typical organometallic compounds could not give rise to a complex of type III since no unshared electrons are available.

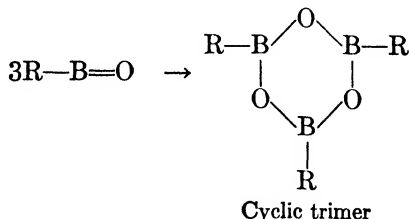
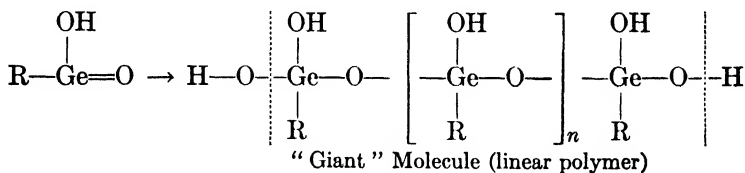
In their behavior toward the free halogens, there is a certain resemblance between the alkyl derivatives of the elements of Group V and Group IV. Phosphorus, arsenic, and antimony form halides of the type R_3PCl_2 , R_2PCl_3 , and RPCl_4 , which are generally stable enough to be isolated. Upon warming, they tend to undergo dealkylation and yield products similar to those obtained by the action of halogens on the alkyl derivatives of the elements of the earlier groups. The normal form of the pentavalent chlorides of the fifth group elements is probably



that in which the central atom is 5-covalent; their stability relative to the corresponding compounds of the earlier groups is due to the higher effective nuclear charge of the central atom. The bismuth alkyls are decomposed directly by halogens, but the tri-aryl derivatives form stable, crystalline dihalides of the type R_3BiCl_2 .

Hydrolysis of the 5-covalent dihalides gives oxygen compounds in which the central atom reverts to an octet. The central atom is undoubtedly only 4-covalent in the normal forms of the arsine-oxides, arsinic, and arsonic acids (and in the corresponding derivatives of phosphorus and antimony).

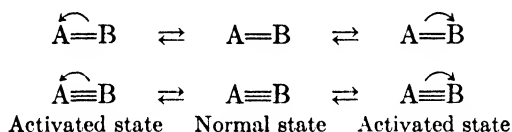
Multiple Covalent Bonds. When there are not enough electrons in a molecule to provide each atom with its stable octet by the process of forming single covalent bonds, two contiguous atoms may share a second or third pair of electrons. The extent of this sharing is by no means so complete or unambiguous as in the single bond; furthermore, the ability to share a second or third pair is almost entirely limited to carbon, nitrogen, and oxygen.¹ This property appears to be associated with the helium configurations of these atomic kernels; the "double bonds" of phosphorus, sulfur, and other elements of Groups V, VI, and VII, outside the first period, are usually coördinate links. The behavior of systems represented for convenience by formulas such as SiO_2 , $R-SiO_2H$, $R-GeO_2H$, etc., is consistent with the view that these substances are actually polymerized in the normal state and would be more accurately represented as "giant" molecules. In the case of boron in the alkyl boric oxides, $R-B=O$, the polymerization gives a cyclic trimer analogous to the trimeric aldehydes.⁶⁸



⁶⁸ Johnson and Snyder, *Organic Chemistry Symposium*, Rochester (1935); see, also Kinney and Pontz, *J. Am. Chem. Soc.*, **58**, 197 (1936).

Lewis¹ pointed out that the sharing of more than one electron pair by two atoms represents, because of the mutual repulsion of the nuclei, a point of weakness or condition of strain in the molecule, and this tends to keep the system from settling into a state of high stability and low electron mobility. Furthermore, the formation of a multiple bond is accompanied by a diminution of the internuclear distance; this amounts to about 10 per cent for a double bond and 20 per cent for a triple bond. He drew the conclusion that the properties of substances with multiple bonds are due to an extent of sharing of two or three electron pairs which is probably less than that indicated in the usual graphic formulas; but the sharing must be assumed to have some physical reality in order to account for the existence of geometrical isomerism and similar steric phenomena.

True covalent multiple bonds of the types $A=B$ and $A\equiv B$ are regarded as capable of existing in an inactive form in which both atoms have a valence octet, and a reactive form in which electromeric displacement of an electron pair creates an electron deficit in the valence shell of one atom (an open sextet) and an increased electron mobility in the other.⁶⁹ The typical states of a double and triple bond may be represented in the following manner (see, also, p. 1618):



The symmetrical active forms that would result from a rupture of the binding pair would give each atom only seven electrons. It seems quite improbable that this mode of activation is significant in the typical reactions of unsaturated systems. Lewis has expressed the view that the pairing of electrons ("rule of two") is even more fundamental than the octet rule, and that a substance containing unpaired electrons would be far more reactive than ethylene actually is.

The active forms are assumed to be extremely mobile and capable of only momentary existence, so that their concentration is always small; owing to the reversibility of the electromeric displacement there is equilibrium between the active and inactive forms. If A and B are different atoms, then, in the activation process, the atom having the higher effective nuclear charge will tend to retain the electron pair and the atom having the smaller nuclear charge will become the deficient atom in the active form. The direction of addition of unsymmetrical addenda

⁶⁹ Carothers, *ibid.*, 46, 2226 (1924).

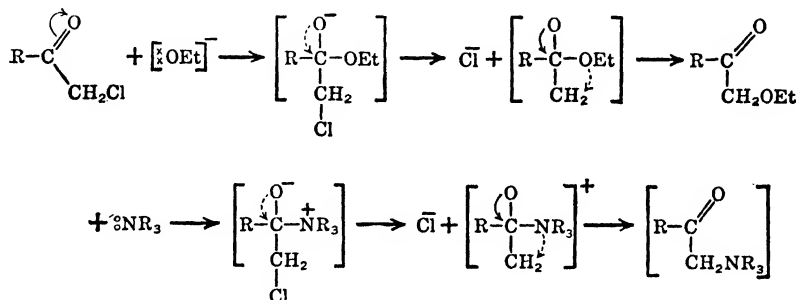
(HX, RMgX, etc.) will be highly selective and will be determined essentially by the electronic configuration of the dominant active form,⁶⁹



Since the two atoms remain attached by one covalent link (two in the triple bond) the deficient atom never gets far away from the displaced electron pair.

The electromeric displacement will affect the remaining covalent links (shared electron pairs) and the mobility of unshared electrons pairs in the entire system. The displacement $A \overset{\curvearrowright}{=B}$ will increase the donor activity of B and will have a dynamic effect of electron-release in the covalent links of B with a substituent. This dynamic influence can be effective in chemical reactions only through unshared electron pairs or other multiple bonds in the substituent; it can interfere with the acceptor activity of B or its substituents only by promoting a different course of reaction. Electron withdrawal by B will increase the acceptor activity of A and the atoms or radicals attached to A, and will facilitate their reactivity toward a donor reagent. Thus, a carbonyl group will increase the proton-escaping tendency of the system into which it is introduced; it enhances the ease of substitution in saturated systems and gives a more definite orientation to the substitution process ($\alpha > \beta > \gamma$, etc.).

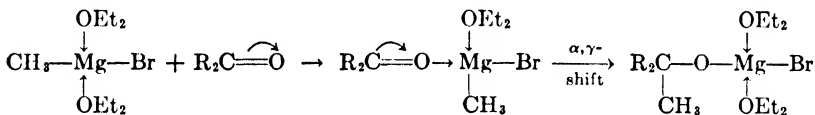
It must be noted that the electron attraction of a carbonyl group (or other multiple bond) does not impede the separation of an anion from the alpha carbon atom in a system such as $Cl-CH_2-A \overset{\curvearrowright}{=B}$; in fact, it can facilitate the elimination of the potential anion (i.e., replacement by another) by an indirect process such as that indicated below.



In an *alpha*-halogenated ketone the electromeric effect of oxygen, and the transmitted inductive effect due to the halogen, enhance the electron deficit of the carbonyl carbon. Under appropriate conditions

the deficient carbon will coördinate with a donor, such as trimethylamine or an ethoxide anion, to form a neutral complex or a complex anion. In either case the oxygen has a high residual negative charge resulting from the acquisition of the electron pair of the double bond; the electromeric effect will tend to be reversed and the elimination of halide ion from the complex is facilitated. As this occurs the entering donor center becomes linked to the alpha carbon.* The effect of the reversed electromeric displacement will diminish rapidly as additional CH₂ groups intervene between the halogen and the carbonyl group.

In many instances, reactions of the typical systems such as C=N, N=O, and C=O, involve a preliminary coördination in which nitrogen or oxygen acts as a donor; this process facilitates withdrawal of the electron pair from the adjacent atom in the link and also increases the mobility of the potential donor center in the reagent molecule. The reactions of these systems with Grignard reagents²⁶ has been interpreted in this way (p. 1649); the normal reactions involve an α,γ -migra-



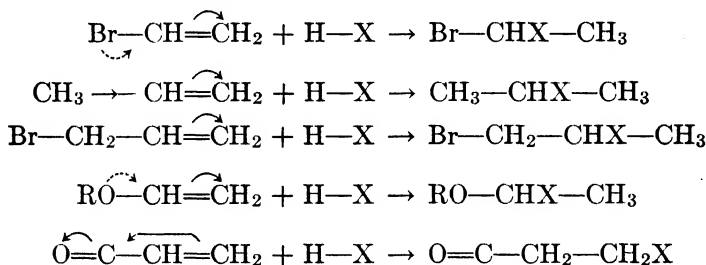
tion of the potential organic anion to the deficient atom of the unsaturated system. The fact that ethylenic and acetylenic bonds do not react with Grignard reagents is indicative of the importance of unshared electron pairs of nitrogen and oxygen in the activation mechanism.

The extent of electromeric displacement in the systems A=B and A \equiv B, under a given set of conditions, will increase as the opposed nuclear charges become greater and for a given compound will be influenced by such factors as the temperature, concentration, characteristics of the medium, catalysts, etc. This permits an explanation of the difference in reactivity of different multiple bonds under the same conditions, and of the same multiple bond under different conditions.⁶⁹ The susceptibility of a system to perturbing influences of substituents (internal factors) and of external factors will increase as the difference in opposed nuclear charges becomes smaller, and therefore reaches a maximum when A and B are the same element. In systems such as R_A-HC=CH-R_B and R_A-N=N-R_B, the configuration of the active form will depend upon the relative influences of the substituents R_A and R_B, and both

* The formulas given above do not take into account the intervention of the cation, the possibility of chelation within the complex, and other influences which may play an important part in certain cases. It is obvious that if the group R can itself be eliminated as an anion, as in Cl-CO-CH₂Cl, the complex anion indicated above would yield an ester instead of the ethoxy ketone.

active forms may result. In general these systems exhibit a definite orientation in their addition reactions; the process of activation involves the interaction of polarization and polarizability effects of the substituent and the multiple link under consideration.

The inductive effect (electron-attraction) of the hetero atoms nitrogen, oxygen, and halogens, in their links with carbon, will facilitate a reaction involving the enhanced acceptor activity of 1-covalent hydrogen in the attached system, but their electromeric effects of electron-release ($-E$) will foster a dynamic displacement in the opposite direction (see Hetero-enoid Systems). Consequently, in the orientation of addition reactions only the $-E$ effect will be significant. The inductive effect of alkyl groups (electron-release relative to hydrogen) will also be of assistance in the orientation process. For similar reasons the inductive electron-release of an atom such as boron will be opposed by its capacity to exert a dynamic effect in the opposite direction. The anticipated orientation in the addition of an unsymmetrical reagent is indicated below for several ethylenic systems.



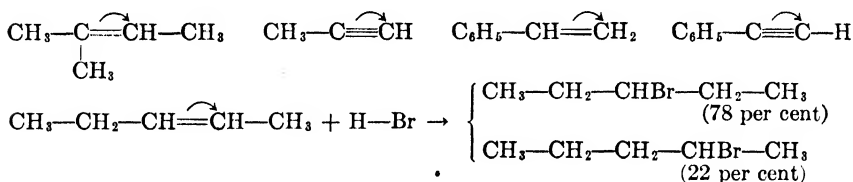
Recent work of Kharasch and his associates⁷⁰ supports the notion that the normal addition of hydrogen bromide to the first three of these systems follows the course indicated, but it was observed that the presence of minute amounts of active substances such as peroxides can suffice to bring about the formation of a large proportion of the isomeric product. The fact that thiophenols usually add exclusively in a direction opposite to that of most reagents illustrates the ability of the addendum itself to influence the process.⁷¹ It is not unlikely that the normal active form dominates in these anomalous cases and the mechanism of the addition process is altered.

The orientation of addition reactions of unsymmetrical olefins and acetylenes involves the relative effects of electron-attraction and elec-

⁷⁰ Kharasch and collaborators, *ibid.*, **55**, 2468, 2521, 2531 (1933); **56**, 712, 1212, 1243, 1425, 1642 (1934); **58**, 57 (1936); *J. Soc. Chem. Ind.*, **54**, 939 (1935).

⁷¹ Posner, *Ber.*, **38**, 646 (1905); Ashworth and Burkhardt, *J. Chem. Soc.*, 1791 (1928); Carothers, *J. Am. Chem. Soc.*, **55**, 2008 (1933).

tron-release in the hydrocarbon substituents. In the aliphatic series the relative electron-attraction diminishes as the number of hydrogen atoms on the *alpha*-carbon is reduced: $\text{CH}_3 > \text{C}_2\text{H}_5 > \text{primary} > \text{secondary} > \text{tertiary}$ groups. All these groups have an effect of electron-release relative to $\text{H}-\text{C}$, consequently the principal active configuration of an unsaturated hydrocarbon will be that in which the electromeric displacement occurs toward the carbon bearing the larger number of hydrogen atoms. The addition of reagents such as $\text{H}-\text{X}$ and $\text{X}-\text{OH}$ will tend to occur in such a way that the donor center of the reagent unites with the carbon atom bearing the larger number of alkyl groups (cf. Markownikoff's rule). The predominating active forms of several hydrocarbon systems are indicated below; the observed



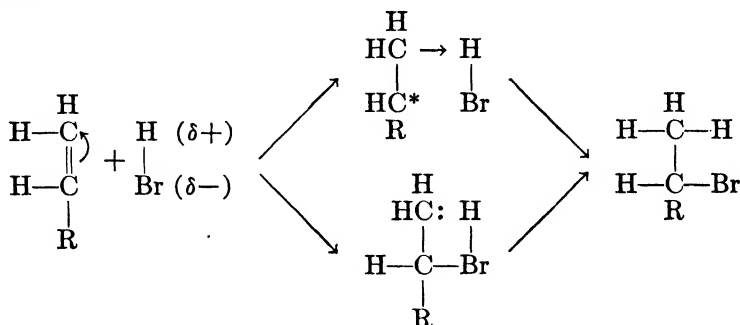
(normal) additions of these systems support the anticipated selective activation. In the case of pentene-2, containing the two similar alkyl radicals methyl and ethyl, it was found that addition of hydrogen bromide gave a mixture of the isomeric alkyl bromides in which the normal product predominates, i.e., 78 per cent of 3-bromopentane to 22 per cent of 2-bromopentane.⁷² In the addition reactions of arylated systems such as styrene and phenylacetylene, the phenyl group has an effect of electron-release relative to hydrogen; the electron-attraction of aryl groups facilitates substitution in the unsaturated side chain but does not contribute to the orientation of addition reactions. Triphenylethylene, for instance, forms with bromine an unstable addition product which decomposes readily to give triphenylvinyl bromide.

Different opinions are held concerning the intimate mechanism of addition reactions of the olefins. Ingold and Robinson consider the addition of hydrogen halides, halogens, and other typical reagents to be initiated by the union of a positive atom (acceptor or electrophilic center) of the addendum with the atom acquiring the unshared electron pair in the activated form of the olefin. Carothers^{69, 73} has expressed the view that the first step is the completion of the electron deficit in the valence shell of the positive atom in the active form of the olefin, by means of an unshared electron pair of the addendum. So far as the

⁷² Lucas and Moyse, *ibid.*, **47**, 1459 (1925).

⁷³ Carothers and Berchet, *ibid.*, **55**, 1628 (1933).

addition reactions of simple olefins are concerned, the same final product would be anticipated from either view of the intimate mechanism of the process.



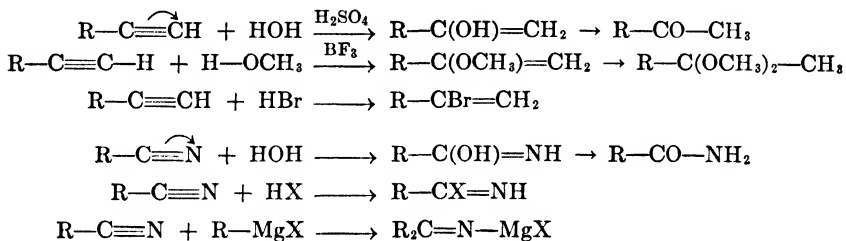
Olefins and carbonyl compounds exhibit a rather definite contrast in their behavior toward chemical reagents (p. 542). Olefins add halogens, halogen acids, nitric and sulfuric acids, ozone, per-acids ($\text{R}-\text{CO}_3\text{H}$), and oxyhalogen acids; they do not react with ammonia and its derivatives (RNH_2 , NH_2OH , NH_2-NH_2), alcohols, alkalies, cyanides, and Grignard reagents. The carbonyl compounds, on the other hand, undergo reaction with the latter group of reagents. Largely because of such differences in the facile type of reactivity, Ingold and Robinson regard the olefins as nucleophilic (donors) relative to the reagents, and the carbonyl compounds as electrophilic (acceptors). This view appears to rest upon the assumption that the ability of oxygen to contribute to the activation process by means of its unshared electron pairs is trivial in relation to the reactivity of the carbonyl compounds; but in the activation of an olefin the electronic excess of one carbon atom is assumed to be responsible for the ultimate acceptor activity of the other.

Robinson⁷⁴ considers that for an equal degree of electronic excess or defect, any carbon atom is much more active in the former condition and that the extent of electromeric change ("activation level") normally found in olefins is not sufficient to initiate reaction with a donor reagent. Addition reactions are regarded in this way: "When the anionoid (donor) center is an olefin which begins to donate electrons to an external molecule, the electromeric change will naturally take a further step, and an adequate defect of electrons on the second carbon of the pair is established and real kationoid (acceptor) activity becomes possible." The contribution of external acceptor and donor centers in the activation of olefins and carbonyl compounds may be expected to

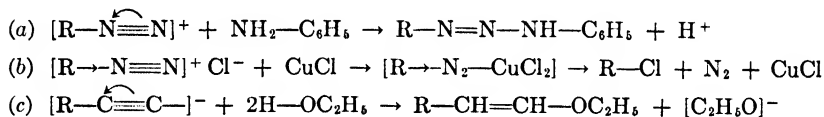
⁷⁴ Robinson, *J. Soc. Dyers Colourists, Jubilee Issue*, 65 (1934).

vary with the environment; in either case the seat of unsaturation in the activated form must be the incomplete valence shell of the deficient atom.

In systems containing covalent triple bonds the theoretical considerations are essentially the same as those applied to the olefins. The addition reactions of acetylenes and of nitriles indicate that the dominant active forms are those anticipated.



The diazonium cations and acetylide anions, $[\text{R}-\underset{\alpha}{\text{N}}\equiv\underset{\beta}{\text{N}}]^+$ and $[\text{R}-\text{C}\equiv\text{C}]^-$, afford examples of the effect of ionic charges upon the configuration of the active forms. In the diazonium cations the large residual positive charge of the 4-covalent nitrogen tends to bring about withdrawal of an electron pair from the multiple link and to create an electron deficit on the β -nitrogen. Under appropriate conditions an active donor may add at this seat of unsaturation and give rise to diazo structures (a). On the other hand, under certain conditions unstable



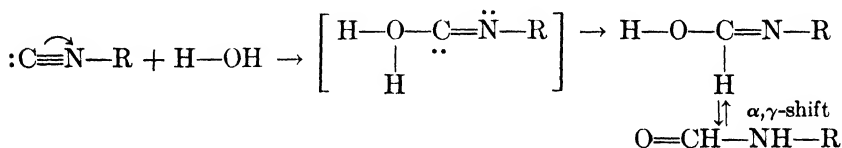
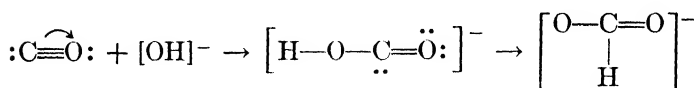
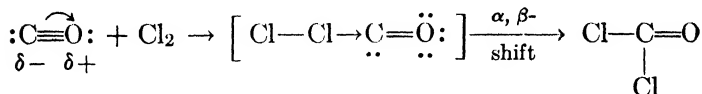
complexes can be formed in which the strong electron-attraction of the positively charged center will facilitate migration of an organic cation (b).

In the acetylide anions the high electron density of the terminal carbon will favor electron-release, and the direction of the electromeric effect may be expected to be the reverse of that in the acetylene itself. Indeed, the orientation of the addition of alcohols to acetylenes is reversed in the presence of strong alkalies (c).

The covalent triple bonds in carbon monoxide and the isocyanides are of an unusual type, owing to the presence of an unshared electron pair on the carbon atom in the normal state of the molecule.⁷⁵ The strong electron-attraction of oxygen or nitrogen will tend to create an

⁷⁵ Hammick, New, Sidgwick, and Sutton, *J. Chem. Soc.*, 1876 (1930); Sidgwick, *Chem. Rev.*, **9**, 77 (1931).

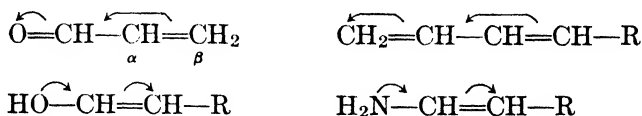
electron-deficit on the carbon atom and facilitate its coördination with a donor center. In the resulting complex an acceptor center may then migrate to the unshared electron pair of the carbon (α , β -shift), and the net result is that both fragments of the addendum become attached to the carbon atom.



The physical properties and reactions of these systems indicate that carbon atoms which have been represented as bivalent in the conventional formulas actually have a complete valence octet made up of a triple covalent link and an unshared electron pair.

POLYFUNCTIONAL ELECTROMERIC SYSTEMS

The union of two or more centers that are capable of taking part in electromeric displacements may give rise to systems of diminished reactivity owing to internal compensation, or of enhanced reactivity resulting from the concurrence of the effects. The direct attachment of one or more unsaturated units, such as $-\text{CH}=\text{CH}-$ or $-\text{C}\equiv\text{C}-$, to an active donor or acceptor center permits a transmission of the activity to another atom in the system. Thus, the acceptor activity of the carbon atom of a carbonyl group or olefin may be transmitted to the β -atom of an attached or unsaturated system; the donor activity of oxygen or nitrogen may be transferred in a similar way to the β -carbon

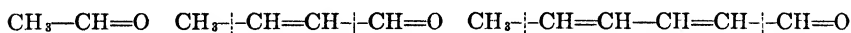


in an attached group. The 1,4-addition reactions of α, β -unsaturated carbonyl compounds and of 1,3-dienes are familiar examples of the first type; the carbon alkylation of ethyl acetoacetate and β -aminocrotonate illustrate the second type.

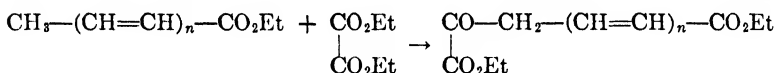
Vinylogous Systems. Fuson⁷⁶ has formulated the following generalization concerning the propagation of the influence of a functional group along an unsaturated chain: When, in a system of the type $X-Y=Z$ or $X-Y\equiv Z$, a structural unit of the type $-(C=C)_n-$ is

$\begin{array}{c} | \quad | \\ -(C=C)_n- \end{array}$

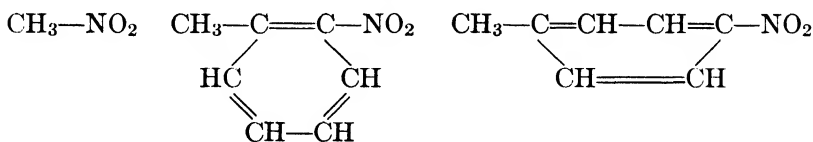
interposed between X and Y, the function of Z remains qualitatively unchanged but that of Y may be usurped by the carbon atom attached to X. It is proposed to term such a group of compounds a vinylogous series, and the members of the series vinylogs of one another. Thus, acetaldehyde, crotonaldehyde, and sorbic aldehyde may be regarded as a vinylogous series, where X is CH_3 , $Y=Z$ is $-CH=O$, and $n = 0$,



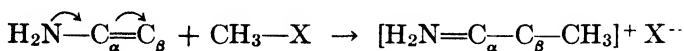
1, and 2. Evidence of the effect of the carbonyl group on the terminal methyl groups in crotonaldehyde and sorbic aldehyde is shown by their ability to undergo aldol condensations forming the higher members of the series. Likewise, ethyl crotonate and sorbate undergo condensation with ethyl oxalate in a manner similar to the reaction of ethyl acetate.



In disubstituted aromatic derivatives of the type $A-C_6H_4-B$, the *ortho* and *para* compounds will have a vinylogous relationship to the system $A-B$, but the *meta* isomer will not be a vinylog of $A-B$. Thus, the methyl group of *o*- and *p*-nitrotoluene is activated, resembling CH_3-NO_2 , but that of *m*-nitrotoluene is not.



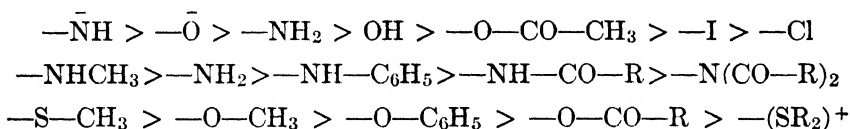
Hetero-enoid Systems. The combination of a hetero atom such as nitrogen, oxygen, or sulfur (in their lower covalent states) with an unsaturated system by means of a single link is termed by Robinson a hetero-enoid system. In these structures the hetero atom tends to increase its covalence with the α -carbon of the unsaturated system, and as a result the donor activity (seat of attack for acceptor reagents) may be transmitted to the β -carbon atom.



⁷⁶ Fuson, *ibid.*, **16**, 1 (1935); see, also, Chap. 6, p. 544.

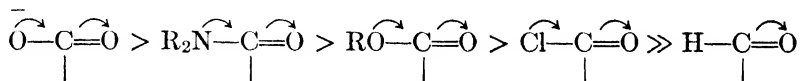
Activation of the *o*- and *p*- positions of the aromatic ring toward acceptor reagents, by substituents such as —OH , —OR , —NH_2 and the halogens, may be attributed to the transmission of the donor activity of these hetero atoms.

The effectiveness of the hetero atoms in increasing the degree of polarization of the β -carbon varies inversely with the number of unshared electrons ($\text{N} > \text{O} > \text{halogens}$) and the effective nuclear charge ($\text{I} > \text{Br} > \text{Cl}$). As a practical guide Robinson gives the following rule: if a series of bases X—H , X'—H , X''—H , is arranged in order of diminishing proton affinity then the systems X—C=C , X'—C=C , X''—C=C , are arranged in order of diminishing polarization and of diminishing donor reactivity exhibited by the carbon atoms. Negatively charged groups as in the phenoxide and enol anions will occur at the top of the scale of effectiveness, but the participation of the hetero atom in some other conjugated electromeric system (involving its available electrons) will diminish its effectiveness. The order of effectiveness will therefore be:



The heterocycles of aromatic type (p. 62) such as pyridine, thiophene, furan, and pyrrole may be regarded as hetero-enoid cycles.

Neutralized Systems. The union of a donor and acceptor center may be expected to result in a diminished reactivity of the system owing to internal compensation. This effect is evident in the union of the carbonyl group with donor systems such as —OH and —NH_2 . In a series of systems of the type X—C=O , when Y is kept constant, the strength of the internal effect will depend upon the electron-release of X, and will correspond to the following sequence:

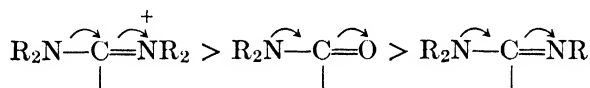


In the carboxylate ion the transference is equivalent to half an electron, since the mesomeric form of the anion is symmetrical with respect to the oxygen atoms.

The transition for the carboxylate anion involves merely the *displacement* of an ionic center, but that for formally neutral systems (amides, esters, acyl halides) requires the *creation* of an electrical dipole. The mesomeric effect is therefore much less than that required for a

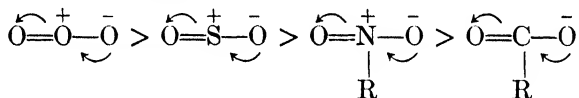
completed interchange. Physical evidence indicates that the order of magnitude of the mesomeric polarization of formally neutral systems is actually about one-tenth of that required for complete polarization. The effect of the whole group $X-C=Y$ upon a system attached at C and the neutralization effect within the group are opposed to each other. Consequently the permanent positive polarization (and the acceptor activity) of the carbon atoms in these systems is the reverse of the above sequence.

If X remains constant and Y is varied, the extent of internal neutralization will be determined by the $+T$ effect of Y. This may be illustrated by the following series:



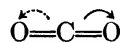
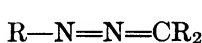
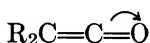
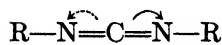
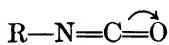
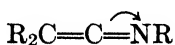
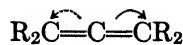
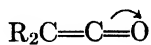
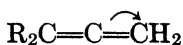
In this instance the acceptor activity of the carbon atom follows the same sequence and is not reversed, since the effect of R_2N- remains constant.

The effect of variations in the central atom may be shown by a comparison of structures represented by the general formula $X=Y-X$, where X is oxygen and Y is carbon, nitrogen, sulfur, or oxygen. In this series the acceptor activity of the central atom increases as its effective nuclear charge becomes greater.



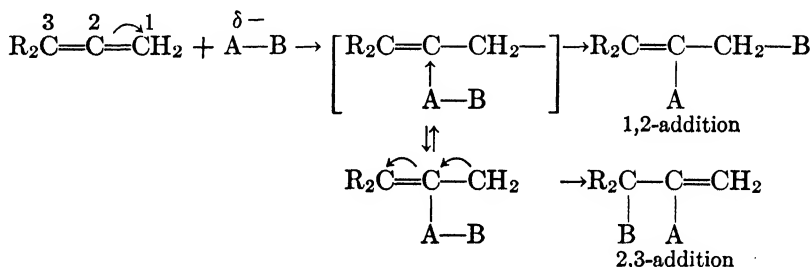
In other neutralized systems of the form $X=Y-Z$, the general principles outlined above may be used to judge qualitatively the relative reactivity of a series of related compounds.

1,2-Dienoid Systems. The 1,2-dienes (allenes) and related compounds containing doubly linked nitrogen and oxygen may be written in the general form $X=Y=Z$. These systems may be considered from the standpoint of the availability of the electron pairs of the multiple links and of unshared electron pairs on the atoms X and Z. The trend of the electron displacements may be considered conveniently by comparing types in which one or two of the components of the systems remain constant. In the first series indicated below, where $X=Y$ is $R_2C=$, the polarization of Z (and the electron deficit of Y) increases from the allenes to the ketenes. The internal effect of neutralization due to a compensating mesomeric electron-release by X must be extremely small since X does not have an unshared electron pair.

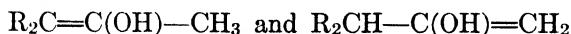


In the principal active form the atom Z withdraws an electron pair of the link $\text{Y}=\text{Z}$, giving rise thereby to an acceptor center at Y and a donor center at Z. The reactivity of these systems toward donor reagents will increase as the effective nuclear charge of Z becomes greater.

In their reactions with unsymmetrical reagents the donor center of the reagent will become attached at Y but the acceptor center of the reagent may become linked at either X or Z, owing to the intervention of electromeric shifts in the course of reaction.



The fact that the allenes of type $\text{R}_2\text{C}=\text{C}=\text{CH}_2$ yield exclusively methyl ketones upon hydration indicates a marked selectivity in the point of attack of the donor center of the reagent. The addition of hydrogen bromide leads to a mixture of isomeric 2-bromo olefins which may arise by fixation of the acceptor center of the reagent in the 1- or 3-positions.⁷⁷ The absence of corresponding isomers in the hydration reaction may be attributed to the fact that 1,2- or 2,3-addition of water would merely give isomeric enol forms of the methyl ketone.



Ingold has pointed out that a number of systems of the general form $\text{X}=\text{Y}=\text{Z}$ contain systems capable of a mesomeric state owing to the presence of an unshared electron pair on the atom X or Z. The alternative structure in this case would contain a triple link, and would have the general form $\text{X}-\text{Y}=\text{Z}$. The second series given above includes the ketenes, isocyanates, and aliphatic diazo compounds as characteristic examples. The ketenes and aliphatic diazo compounds

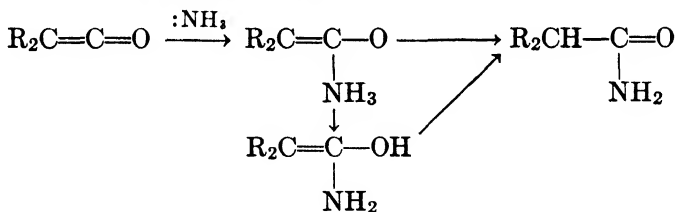
⁷⁷ Bouis, *Ann. chim.*, [10] 9, 402 (1928).

illustrate systems in which only one of the terminal atoms can furnish an unshared electron pair. In the azides and isocyanates either terminal atom can furnish an unshared pair, and two different mesomeric polarizations are possible (Table XIV).

TABLE XIV
MESOMERIC EFFECTS IN STRUCTURES $X=Y=Z$

Type	Formula	Mesomeric Polarization	Alternative Structure
Ketenes.....	$R_2C=C\overset{\curvearrowright}{=O}$	$R_2C\overset{\curvearrowleft}{=}\overset{\curvearrowright}{C=O}$	$R_2\ddot{C}-C\equiv O$
Diazo compounds...	$R_2C=N=N$	$R_2C\overset{\curvearrowleft}{=}\overset{\curvearrowright}{N=N}$	$R_2\ddot{C}-N\equiv N$
Azides.....	$R-N=N=N$	$R-\overset{\curvearrowleft}{N}=\overset{\curvearrowright}{N}=\overset{\curvearrowright}{N}$ (major) $R-\overset{\curvearrowright}{N}=\overset{\curvearrowleft}{N}=\overset{\curvearrowright}{N}$ (minor)	$R-\ddot{N}-N\equiv N$ $R-N\equiv N-\ddot{N}$
Isocyanates.....	$R-N=C\overset{\curvearrowright}{=O}$	$R-\overset{\curvearrowleft}{N}=\overset{\curvearrowright}{C=O}$ (major) $R-\overset{\curvearrowright}{N}=\overset{\curvearrowleft}{C=O}$ (minor)	$R-N\equiv C-\ddot{O}$ $R-\ddot{N}-C\equiv O$
Carbon dioxide....	$O=C\overset{\curvearrowright}{=O}$	$\overset{\curvearrowleft}{O}=\overset{\curvearrowright}{C=O}$	$\ddot{O}-C\equiv O$

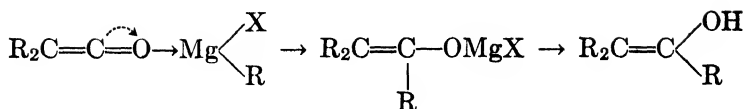
The addition of alcohols and amines to ketenes and isocyanates may be regarded as a direct combination of the donor center of the addendum at the carbonyl carbon. The postulation of an enol form of the intermediate is unnecessary since the acceptor center of the addendum may shift directly to give the more stable form (ester or amide).



The addition of halogen acids may occur by a similar mechanism or may be initiated by coördination of the carbonyl oxygen with an acceptor center of the addendum. The fact that ketenes generally react more rapidly with active donor addenda than with acceptors may be considered to favor the former view.

The reaction of ketenes and isocyanates with Grignard reagents has been studied by Gilman,⁷⁸ and in these cases there is undoubtedly a preliminary coördination of the carbonyl oxygen.

⁷⁸ Gilman and collaborators, *J. Am. Chem. Soc.*, **42**, 1010 (1920); **46**, 493 (1924).



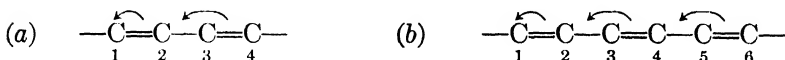
Diphenylketene on treatment with phenylmagnesium bromide, and subsequent hydrolysis, was found to give the stable enol form of diphenylacetophenone (pp. 430 and 573). A similar mechanism was demonstrated experimentally for the isothiocyanates.

Although the existence of alternative structures of the type $R_2\ddot{C}-C\equiv O$ (Table XIV) for the ketenes has not been verified experimentally, the fact that acyl substituted ketenes show a strongly diminished acceptor activity suggests an internal neutralization of the following type:

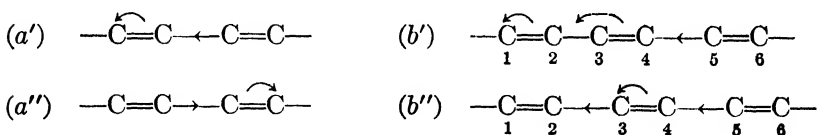


In these structures the strong $+E$ effect of the α,β -unsaturated carbonyl system opposes the normal electromeric polarization of the ketene carbonyl group and reinforces the mesomeric polarization. In the resulting structure (II) the electronic excess is transmitted to the carbonyl oxygen and the resonance between I and II has the effect of diminishing the reactivity toward an external donor molecule.

1,3-Dienoid and Polyenoid Systems. The union of two or more multiple bonds in a 1,3 or 1,3,5 relationship gives rise to an interaction (conjugation) within the system. The extent of this interaction varies over a wide range, and the behavior of the systems is influenced by internal and external factors. Conjugate addition at the 1,4- or 1,6-positions arises from the ability of the system to transmit an electronic deficit, resulting from the electromeric polarization of one multiple bond, to the terminal atom of the second or third multiple bond (a and b).

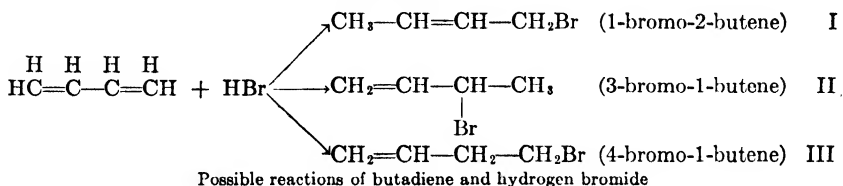


Aside from the conjugate active form the 1,3-diene may exist in non-conjugate active forms (a' , a'') and the 1,3,5-triene in partially conjugate or non-conjugate active forms (b' , b'').



It has sometimes been assumed⁷⁹ that typical 1,4-addition reactions of the dienes involve a completed 1,2-addition followed by allylic rearrangement. The interpretation of much of the experimental evidence bearing upon this point is uncertain owing to the facile interconversion of the isomeric 1,2- and 1,4-adducts, and the effects of oxygen and peroxides.⁸⁰ Nevertheless, there is now definite evidence that chlorine⁸¹ and halogen acids²⁸ yield 1,4-adducts directly in certain instances, and the hypothesis that they necessarily arise by allylic rearrangement must be abandoned. Reagents such as halogens and halogen acids usually produce a mixture of 1,2- and 1,4-adducts, whereas perbenzoic acid⁸² yields only 1,2-adducts (substituted ethylene oxides) and maleic anhydride only 1,4-adducts⁸³ (p. 578).

Kharasch and his collaborators⁸⁰ have found that in the absence of oxygen and peroxides, and in the presence of an antioxidant, butadiene adds hydrogen bromide at low temperatures to give principally the 1,2-adduct, 3-bromo-1-butene (II). At higher temperatures under the influence of hydrogen bromide, and particularly under the combined influence of hydrogen bromide and peroxides, this product rearranges to I. The addition product obtained in the presence of air or added peroxides is principally I (crotyl bromide). Evidence is lacking to show whether peroxides cause direct formation of crotyl bromide by 1,4-addition of hydrogen bromide to butadiene, or merely rearrange the 1,2-addition product. All that can be said with assurance is that in the absence of peroxides 1,4-addition does not occur *with hydrogen bromide*. A careful but unsuccessful search was made for the third possible addition product, III. Two independent analytical methods indicate that not more than 5 per cent of this substance could have been present in the reaction products.



Considerations of the 1,2- and 1,4-addition of hydrogen and of bromine to butadiene, based upon the quantum mechanics, indicate

⁷⁹ Claisen and collaborators, *J. prakt. Chem.*, **105**, 74 (1922); Gillet, *Bull. soc. chim. Belg.*, **31**, 366 (1922); Ingold, Shoppee, and Thorpe, *J. Chem. Soc.*, 1477 (1926); Burton, *ibid.*, 1651 (1928); Farmer and Scott, *ibid.*, 172 (1929).

⁸⁰ Kharasch, Margolis, and Mayo, *J. Org. Chem.*, **1**, 393 (1936).

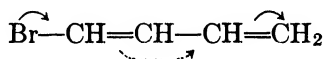
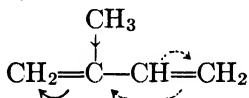
⁸¹ Muskat and Northrup, *J. Am. Chem. Soc.*, **52**, 4043 (1930).

⁸² Pummerer and Reindel, *Ber.*, **66**, 335 (1933).

⁸³ Diels and Alder, *Ann.*, **460**, 98 (1928); **478**, 139 (1930).

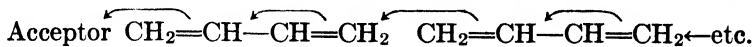
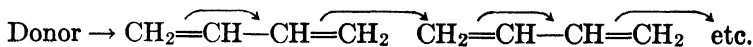
that it is much easier for addition to take place in the 1,4-positions.⁸⁴ This conclusion rests upon the observation that calculated values of the activation energy for the 1,4-reactions are appreciably smaller than those for the 1,2-reactions. The introduction of a substituent may alter the situation and make 1,2-reaction occur more readily.

The degree of electromeric polarization of the 1,3-dienes must be very small or spontaneous polymerization would take place even in the absence of catalysts. Probably effective polarization occurs through the intervention of an active center of the reagent or catalyst. Unsymmetrical substitution will generally enhance the small permanent polarization and will exert a directive effect. In 2-methylbutadiene (isoprene) the $-I$ effect of the alkyl group will favor the development of donor activity at the 1-position, whereas in 1-bromobutadiene the substituent will favor the development of donor activity at the remote position.



The occurrence of this orientation in isoprene is confirmed by the fact that conjugate addition of hydrogen bromide yields 2-methyl-4-bromobutene-2, and that non-conjugate addition reactions yield 1,2- rather than 3,4-adducts.

Robinson has suggested that the polymerization of 1,3-dienes under the influence of a trace of sodium is a chain reaction initiated by the effective polarization of one molecule of the diene resulting from the entry of an electron or electron pair at the acceptor center; the polarized molecule attacks a second molecule of the diene in the same fashion and the process continues until long chains or large rings are formed (p. 1703). Molecular oxygen and peroxides may play a similar role in the initiation of polymerization reactions. It is possible that a chain reaction of opposite type may be initiated by the intervention of an external acceptor, since the diene system is capable of functioning either as a donor or an acceptor.



The aliphatic 1,3-dienes generally show a reactivity greater than that of the simple olefins toward the usual reagents, such as halogens and

⁸⁴ Eyring, Sherman, and Kimball, *J. Chem. Phys.*, **1**, 586 (1933).

halogen acids, and they undergo a number of reactions that are not shown by compounds having an isolated double bond. The characteristic diene reactions include coupling with nitrobenzenediazonium chloride,⁸⁵ facile linear polymerization under the influence of alkali metals, direct addition of alkali metals⁸⁶ and of triphenylmethyl,⁸⁷ and the Diels-Alder reaction with α,β -unsaturated carbonyl compounds⁸³ (p. 593).

Thermochemical data show that the heat of formation of a conjugated system is greater than the sum of the heats of formation of the separate bonds. This departure from strict additivity corresponds to an increase in molecular stability and is interpreted as resonance energy (perturbation energy). Quantum mechanical calculations⁸⁸ have led to values of resonance energies that can be brought into good agreement with those obtained from heats of combustion. The numerical values for several types of conjugated systems, expressed in Calories per mole, are tabulated below. Numbers in italics are calculated values; the remainder are empirical values from thermochemical data.

TABLE XV
RESONANCE ENERGIES OF CONJUGATED SYSTEMS
(PAULING AND SHERMAN)

1,3-Dienes.....	8.0	Benzene.....	37.3	Pyridine.....	43.1
1,3,5-Trienes.....	16.7	Styrene.....	46.1	Pyrrole.....	22.6
1,3,5,7-Tetrenes.....	25.1	Stilbene.....	94.3	Thiophene.....	31.1
Fulvenes.....	15.0	Naphthalene.....	74.7	Furan.....	21.4

The marked effect of conjugation upon the energetics of unsaturated systems is demonstrated by a comparison of the heats of hydrogenation of olefins, dienes, and benzene.⁸⁹ When two double bonds are separated by three single bonds, as in 1,5-hexadiene, there is practically no interaction; the heat of hydrogenation is twice that of a simple olefin of corresponding type. There appears to be a small labilizing effect in the 1,4-diene, and in the 1,2-diene a large labilizing effect. The 1,3-diene instead of exhibiting a labilizing effect of intermediate magnitude actually shows a definite stabilizing effect. Similar effects occur in

⁸⁵ Meyer and collaborators, *Ber.*, **47**, 1754 (1914); **52**, 1472 (1919).

⁸⁶ Ziegler, Orth, and Weber, *Ann.*, **479**, 292 (1930); **504**, 131 (1933).

⁸⁷ Conant and Scherp, *J. Am. Chem. Soc.*, **53**, 1941 (1931).

⁸⁸ Pauling and Sherman, *J. Chem. Phys.*, **1**, 606, 679 (1933).

⁸⁹ Kistiakowsky, Ruhoff, Smith, and Vaughan, *J. Am. Chem. Soc.*, **57**, 876 (1935); **58**, 137, 146 (1936); Conant and Kistiakowsky, *Chem. Rev.*, **20**, 181 (1937).

cyclopentadiene and cyclohexadiene, and an enormous effect arises in benzene. Table XVI gives the heats of hydrogenation (ΔH , in Calories per mole) for several olefins and polyenes, together with the magnitude of the effects of stabilization ($+\Delta Z$) or labilization ($-\Delta Z$).

TABLE XVI
HEATS OF HYDROGENATION OF OLEFINS AND POLYENES

Olefins	$-\Delta H$	Polyenes	$-\Delta H$	ΔZ
$\text{CH}_2=\text{CH}_2$	32.82	1,2-Propadiene	71.28	-14.5*
$\text{R}-\text{CH}=\text{CH}_2$ (mean)	30.20	1,3-Butadiene	57.07	+ 3.3†
$\text{CH}_3-\text{CH}=\text{CH}-\text{CH}_3$ <i>cis</i>	28.57	1,4-Pentadiene	60.79	- 0.4†
<i>trans</i>	27.62	1,5-Hexadiene	60.52	- 0.1†
$(\text{CH}_3)_2\text{C}=\text{CH}_2$	28.37	Cyclopentadiene	50.86	+ 6.3‡
$(\text{CH}_3)_2\text{C}=\text{CH}-\text{CH}_3$	26.92	Cyclohexadiene	55.37	+ 1.8‡
Cyclohexene	28.59	Benzene	49.80	+36.0‡

* Referred to isobutylene.

† Referred to $\text{R}-\text{CH}=\text{CH}_2$.

‡ Referred to cyclohexene.

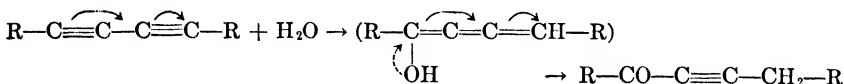
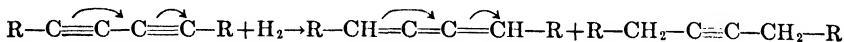
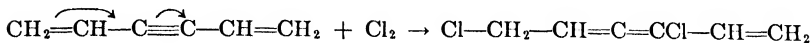
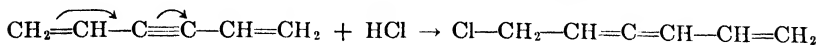
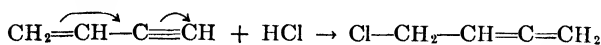
The calculated resonance energy of benzene (Table XV) is in good agreement with the stabilization effect observed by this method, but the calculated value for 1,3-dienes shows a wider deviation from the actual values.

In benzene and similar systems of aromatic character the internal compensation has the effect of facilitating substitution rather than simple addition reactions. However, there is evidence to support the view that substitution reactions of a number of aromatic systems proceed by way of a preliminary addition process (p. 110). Halogen atoms, hydroxyl, and amino groups directly attached to an aromatic ring give rise to hetero-enoid systems in which the electromeric effect ($-E$) of the substituent interacts with the polyenoid system (see Hetero-enoid Systems and Vinylogous Systems). It is possible that a part of the difficulty encountered in developing a satisfactory general theory of aromatic substitution is due to the situation that the reactions can occur either by a direct or an indirect (addition) mechanism.

Triple bonds are capable of taking an active part in conjugated systems (p. 576). The combination of a double and triple bond, as in vinylacetylene, gives rise to a system that undergoes only conjugate addition with halogen acids.⁹⁰ In this instance the orientation of the addition is determined by the triple bond. Divinylacetylene likewise

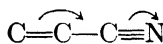
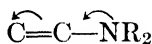
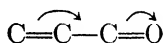
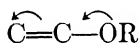
⁹⁰ Carothers, Berchet, and Collins, *ibid.*, **54**, 4066 (1932).

adds chlorine or hydrogen chloride in the 1,4-positions;⁹¹ no evidence of 1,6-addition to divinylacetylene has been observed.



Studies of substituted 1,3-diynes by Grignard and Tcheoufaki⁹² demonstrate that systems containing two triple bonds undergo conjugate addition of bromine and hydrogen bromide. Similarly, catalytic hydrogenation over platinum, and partial hydration, lead to 1,4-adducts.

α,β -Unsaturated Carbonyl Systems and Related Types.* The conjugation of an ethylenic linkage with an unsymmetrical multiple bond of oxygen or nitrogen gives rise to structures of the type $\text{C}=\text{C}-\text{C}=\text{A}$ and $\text{C}=\text{C}-\text{B}=\text{A}$. In the single links of oxygen and nitrogen (heteroenoid systems) the hetero atom exerts a dynamic electron-release, but in their multiple bonds the direction of the electromeric effect is reversed.



Hetero-enoid

Katio-enoid

The essential feature of the α,β -unsaturated carbonyl types (p. 581) is the ability of the system to transfer the seat of acceptor activity to the β -carbon of the ethylenic bond. Consequently, the β -carbon is attacked by the donor center of various reagents, such as ammonia, amines, alcohols, organomagnesium halides, and alkali cyanides, which are without action on simple olefins.

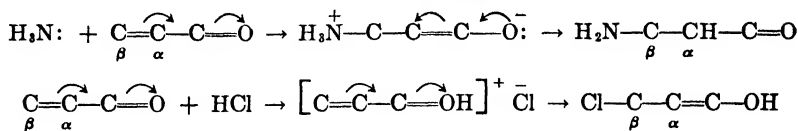
The addition of amines, alcohols, and alkali cyanides to α,β -unsaturated carbonyl systems may be regarded as a direct combination of the donor center of the addendum at the β -carbon, but in the addition of Grignard reagents and halogen acids it is likely that the reaction is initiated by coordination of the hetero atom with an acceptor center of

⁹¹ Coffman and Carothers, *ibid.*, **55**, 2040, 2048 (1933).

⁹² Grignard and Tcheoufaki, *Compt. rend.*, **188**, 527, 1531 (1929).

* These were classified by Robinson originally as "crotonoid" and later as "katio-enoid" systems.

the addendum. The tendency to permanent polarization in the α,β -unsaturated carbonyl systems is necessarily small, owing to the fact



that integral polarization produces an unstable electronic configuration (open-sextet) in the β -position.

Although α,β -unsaturated carbonyl systems show a marked tendency to undergo conjugate addition, there are many instances in which one of the multiple bonds functions independently. The relative amounts of 1,2- and 1,4- adducts are influenced by substituents in the conjugated system (internal factors) and also by the nature of the addendum and the environment (external factors). Kohler⁴⁶ has pointed out that nearly all reactions involving 1,2-addition to carbonyl are reversible, whereas the products formed by 1,4-addition (except with organometallic compounds) undergo rearrangement into saturated carbonyl compounds that are still capable of undergoing 1,2-addition. Under these conditions the products ultimately isolated do not represent the relative rates of 1,2- and 1,4-addition but merely the relative stability of the substances or the position of equilibrium in the particular environment.

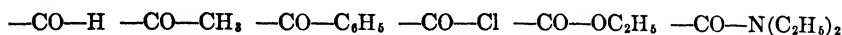
TABLE XVII

ADDITION OF $\text{C}_6\text{H}_5\text{MgBr}$ TO α,β -UNSATURATED CARBONYL SYSTEMS

	Per Cent 1,4-Adduct		Per Cent 1,4-Adduct
$\text{CH}_2=\text{CH}-\text{CO}-\text{H}$	0	$\text{CH}_2=\text{CH}-\text{CO}-\text{C}_6\text{H}_5$	100
$\text{CH}_3-\text{CH}=\text{CH}-\text{CO}-\text{CH}_3$	40	$\text{CH}_3-\text{CH}=\text{CH}-\text{CO}-\text{C}_6\text{H}_5$	100
$\text{C}_6\text{H}_5-\text{CH}=\text{CH}-\text{CO}-\text{CH}_3$	12	$\text{C}_6\text{H}_5-\text{CH}=\text{CH}-\text{CO}-\text{C}_6\text{H}_5$	94
$\text{C}_6\text{H}_5\text{CH}=\text{CH}-\text{CO}-\text{C}_2\text{H}_5$	40	$(\text{C}_6\text{H}_5)_2\text{C}=\text{CH}-\text{CO}-\text{C}_6\text{H}_5$	0
$\text{C}_6\text{H}_5\text{CH}=\text{CH}-\text{CO}-\text{OC}_2\text{H}_5$	50	$(\text{CH}_3)_2\text{C}=\text{CH}-\text{CO}-\text{CH}_3$	0
$\text{C}_6\text{H}_5\text{CH}=\text{CH}-\text{CO}-\text{NR}_2$	90	$\text{C}_6\text{H}_5-\text{C}\equiv\text{C}-\text{CO}-\text{C}_6\text{H}_5$	0

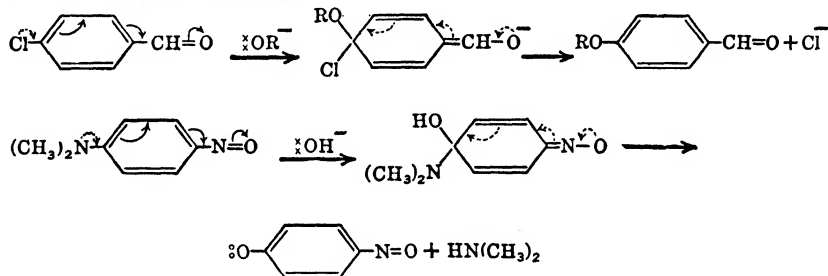
The addition of Grignard reagents to α,β -unsaturated systems affords a comparison of the relative rates of 1,2- and 1,4-addition, as the reactions are not reversible and both adducts are stable. The reactions have been studied extensively by Kohler and his collaborators and a few of their data are shown in Table XVII. The results indicate that 1,4-addition increases as the reactivity of the carbonyl system toward R-MgX diminishes. Thus, in the series shown below the reactivity toward Grignard reagents diminishes from $\text{R}-\text{CO}-\text{H}$ to $\text{R}-\text{CO}-\text{N}(\text{C}_2\text{H}_5)_2$, but the tendency of the α,β -unsaturated carbonyl

systems to undergo 1,4-addition diminishes in the opposite direction, from $R-CH=CH-CO-N(C_2H_5)_2$ to $R-CH=CH-CO-H$.



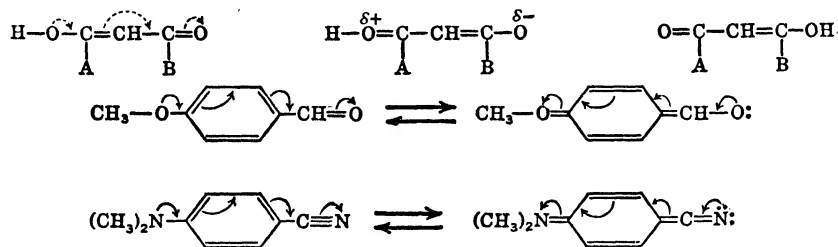
The effect of a hydrocarbon group attached in the β -position is relatively slight, but the presence of two hydrocarbon groups on the β -carbon impedes 1,4-addition. It is of interest to note that an acetylenic system such as $C_6H_5-C\equiv C-CO-C_6H_5$ does not undergo conjugate addition of Grignard reagents.

Robinson has pointed out that the enhanced acceptor activity of the terminal carbon atom in "katio-enoid" structures facilitates the exchange of anions in these systems; the increased activity of aryl halides containing a nitro or carbonyl group in the *ortho*- or *para*-positions and the hydrolysis of *p*-nitrosodimethylaniline are explained by the following mechanisms:



It is evident that the same groups in the *meta*-position will not function in a similar manner owing to the inability of the system to transfer an electronic deficit to the *meta*-position.

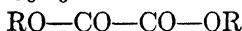
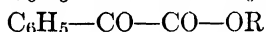
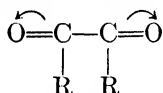
The presence of hydroxyl or amino substituents in the β -position of an α,β -unsaturated carbonyl group gives rise to an internal compensation resulting from the effect of dynamic electron-release within the hetero-enoid system. The dynamic isomerism of unsymmetrical enols, reduced carbonyl activity of *p*-methoxybenzaldehyde and *p*-dimethylaminoaryl ketones, and the relative inactivity of the corresponding nitriles toward $RMgX$, are typical examples.



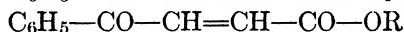
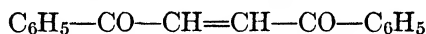
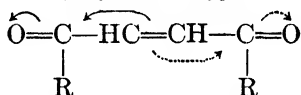
The order of effectiveness of various *p*-substituents in bringing about internal neutralization is the same as that given under hetero-enoid systems (p. 1680). It must be recognized that these dynamic effects act largely to reduce the rate of carbonyl reactivity and in this way may favor an alternate course of reaction, such as replacement of the β -substituent (*cf.* preceding paragraph).

Quinonoid Systems. Two carbonyl groups, or similar types, united directly or by means of an intervening ethylenic system, give rise to *ortho*- and *para*-quinonoid structures. Owing to the tendency of the two groups to promote electromeric changes in opposed directions, these systems are highly reactive and the units frequently function independently in their reactions.

1,2-Quinonoid types

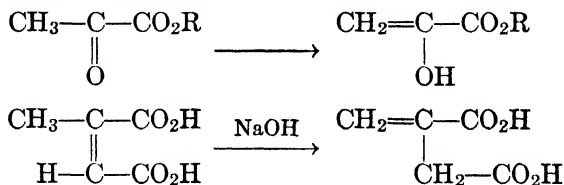


1,4-Quinonoid types



In addition to the true *ortho*- and *para*-benzoquinones, this group includes simple 1,2-dicarbonyl compounds (glyoxal, biacetyl, benzil, ethyl oxalate, and α -ketoic acids) as well as 1,2-dicarbonyl derivatives of ethylene (dibenzoyl ethylene, benzoylacrylic acid, maleic acid, and citraconic acid).

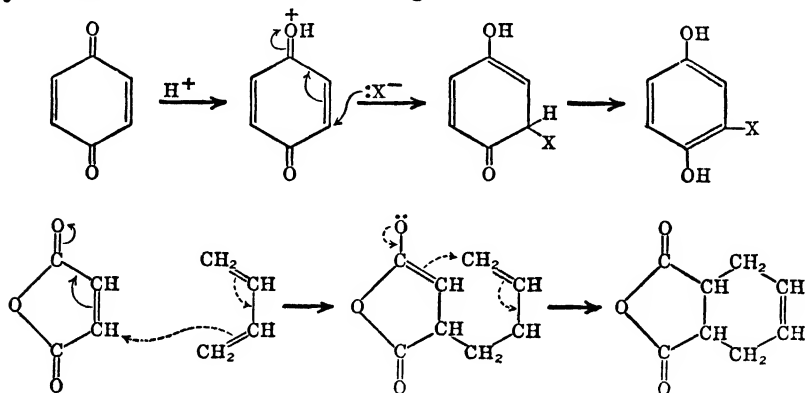
The strong activating influence of the $-\text{CO}-\text{CO}_2\text{R}$ group on an adjacent methylene group, and the citraconic-itaconic acid rearrangement, may be regarded as manifestations of the tendency of a quinonoid system to revert to one in which the tension of the opposed electromeric effects has been relieved.



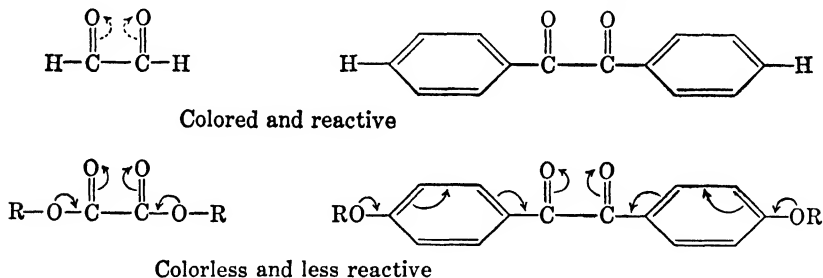
The enhanced reactivity of quinonoid systems is illustrated by the facile addition of acids to *p*-benzoquinone, addition of 1,3-dienes to quinones and to maleic anhydride (Diels-Alder reaction), and the conversion of aryl 1,2-diketones into benzilic acids by means of alkalis.

The addition of acids is probably initiated by fixation of a proton at the carbonyl group, resulting in the development of an active acceptor

center in the β -position. Combination of a donor reagent at this point is followed by isomerization to a substituted hydroquinone. However, the Diels-Alder reaction, benzilic acid rearrangement, and condensation reactions occurring in alkaline media may be regarded as a direct attack by an active donor center of the reagent.

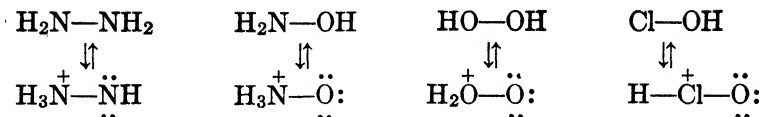


Robinson has given an interesting example of the effect of neutralized systems on the 1,2-diketone group. Glyoxal is a colored substance (yellow solid, green vapor) and is highly reactive as a carbonyl com-

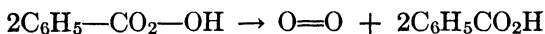


pound, whereas ethyl oxalate is colorless and far less reactive. The same relationship holds true for benzil and *p,p'*-diethoxybenzil; the former is colored and reactive, the latter is colorless and much less reactive. This analogy affords a striking illustration of the principle of vinylogy (pp. 544, 1679).

Peroxidic Systems. The direct union of amino and hydroxyl groups with each other, or with halogen atoms, gives rise to discordant systems of type opposite to the quinones. The former develop an active donor center and the latter an acceptor center. In the simple peroxidic systems such as hydrogen peroxide, hydroxylamine, and hypochlorous acid, α,β -proton migration may give rise to a tautomeric relationship (see Dyad Systems, p. 1705).

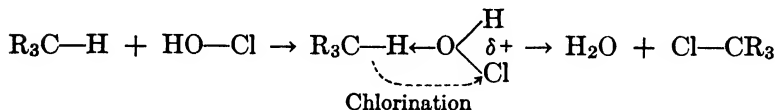
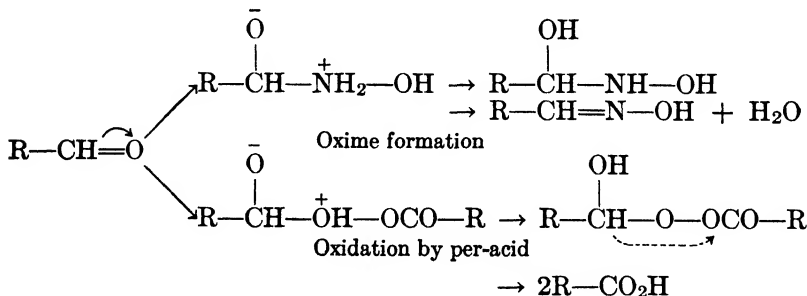


A similar dynamic isomerism is possible in the partially substituted derivatives such as mono-, di-, and trisubstituted hydrazines, N-substituted hydroxylamines, and per-acids. This group of compounds is characterized by an ability to act either as oxidizing or reducing agents, according to the nature of the environment; many of them undergo disproportionation reactions involving mutual oxidation and reduction.



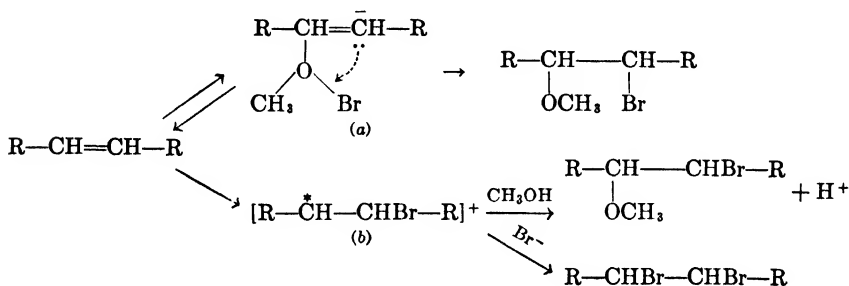
The disproportionation of hydrogen peroxide into water and molecular oxygen is paralleled in the organic derivatives by the corresponding reaction of perbenzoic acid and by the conversion of hydrazobenzene into azobenzene and aniline.⁹³ The facile conversion of β -phenylhydroxylamine into *p*-aminophenol and of hydrazobenzene into benzi-dine, under the influence of strong acids, affords a further illustration of the instability of these systems. The fact that rearrangement of peroxidic systems is brought about by acids, and quinonoid systems by alkalis, is a direct consequence of their respective donor and acceptor activity.

Typical additive processes such as the formation of oximes and hydrazones, and oxidation by per-acids, are probably initiated by attack of an external acceptor center by an unshared electron pair of the peroxidic system.

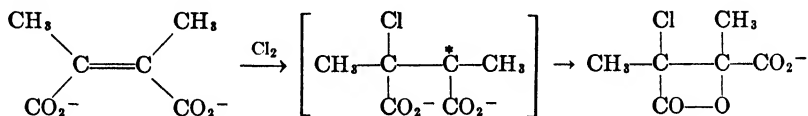


⁹³ Wieland, *Ber.*, **48**, 1098 (1915); see, also, Kenner and Knight, *Ber.*, **69**, 341 (1936).

The view that addition of hypohalous acids and alkyl hypochlorites to olefins proceeds by a similar mechanism (a) is not generally accepted. Recent kinetic studies of addition reactions of stilbene⁹⁴ indicate a step-wise process (b) in which an active intermediate is produced by combination of a donor center of the olefin with an acceptor of the addendum ("positive bromine"). The composition of the adduct is determined by competitive reactions of the labile intermediate with an active donor center of the environment ($\text{CH}_3\text{—OH}$ or Br^-).



It is difficult to reconcile this formulation (b) with the observation that stilbene and isostilbene yield different stereoisomeric adducts, since true carbonium cations are considered to be configuratively unstable⁹⁵ and would lead to identical stereoisomers from the *cis*- and *trans*-stilbene. The formulation of a carbanion intermediate appears to afford a more satisfactory explanation of the relevant experimental evidence.⁹⁶ On the other hand, it is difficult to account for the formation of chloro- and bromo- β -lactones by the addition of chlorine and bromine to aqueous solutions of salts of dimethylmaleic and dimethylfumaric acid⁹⁴ without recourse to the hypothesis that the positive fragment of the halogen molecule is added as the first step. Further work in this field will be of considerable interest. There is, of course, no reason to expect that the intimate mechanism of olefinic addition must be the same for all olefins, or for all addenda.



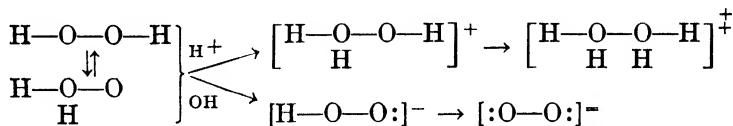
The mechanism of oxidation and reduction by peroxidic structures affords an interesting and difficult problem. Owing to the tautomeric

⁹⁴ Bartlett and Tarbell, *J. Am. Chem. Soc.*, **58**, 466 (1936); **59**, 407 (1937).

⁹⁵ Wallis and Adams, *ibid.*, **55**, 3838 (1933).

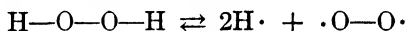
⁹⁶ Ogg, *ibid.*, **57**, 2727 (1935).

character of the typical systems and their interaction with acids, bases, and hydroxylic solvents, a number of reactive species may be involved.



The importance of hydroxylic solvents is indicated by Wieland's observation that peracetic acid does not attack dry acetaldehyde but does attack it in the presence of water; however, this difference is not observed with benzaldehyde. Wieland⁹⁷ considers that hydrogen peroxide acts as a hydrogenating (reducing) agent owing to its ability to decompose into molecular oxygen and monatomic hydrogen, and acts as a dehydrogenating (oxidizing) agent through combination with monatomic hydrogen to form water.

An extension of Wieland's view by Bancroft and Murphy⁹⁸ involves the postulation of a reversible dissociation and the production of an activated form of oxygen. When hydrogen peroxide acts as an oxidizing agent, the active oxygen is assumed to react both with the substance



to be oxidized and with the monatomic hydrogen. They have found that the true electromotive force of hydrogen peroxide in approximately molar acid solutions is about $E_h = +1.16 \pm 0.3$ volts, and in molar potassium hydroxide solutions about $E_h = +0.30 \pm 0.03$ volt. The data indicate that oxidizing and reducing agents having a larger E_h value than hydrogen peroxide (under the given conditions) are reduced by it, and those having a smaller E_h value are oxidized.

Raikow⁹⁹ believes that a substance cannot have the same formula as a reducing agent and an oxidizing agent; he attributes reducing action to the normal form $\text{HO}-\text{OH}$, and oxidizing action to the oxonium structure $\text{H}_2\text{O}-\text{O}$. It is pointed out that reductions by hydrogen peroxide are rapid reactions whereas oxidations are slow; these facts are explained on the assumption that the oxonium tautomer is present in small concentrations and is formed from the normal structure by a slow reaction. This hypothesis does not take into account the circumstance that either tautomer gives rise to the same cation or anion. Nevertheless there

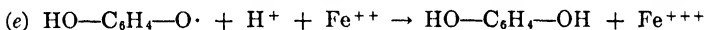
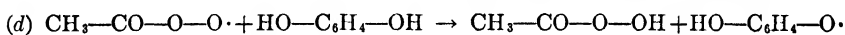
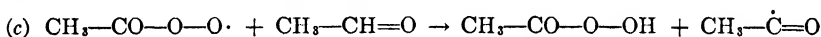
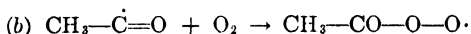
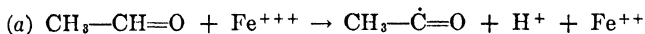
⁹⁷ Wieland, *Ber.*, **54**, 2361 (1921).

⁹⁸ Bancroft and Murphy, *J. Phys. Chem.*, **39**, 377 (1935).

⁹⁹ Raikow, *Z. anorg. allgem. Chem.*, **168**, 297 (1928); **189**, 36 (1930).

is evidence that tautomeric phenomena are involved in the reactions of peroxidic systems, since disubstituted organic peroxides such as dibenzoyl peroxide and diethylperoxide are much less active than perbenzoic acid and ethyl hydroperoxide.

The tautomeric forms of hydrogen peroxide and the monovalent ions possess a donor and an acceptor center, and can enter into reaction by coordination processes. The mode of reaction and observed catalytic effects may be associated with a specific orientation of the coordination mechanism, but the prevalent view is that oxidation-reduction reactions in aqueous solutions involve free radicals and proceed by means of a chain. The mechanism for aldehyde oxidation proposed by Haber and Willstätter,¹⁰⁰ and subsequently modified by others, envisages the following steps: (a) formation of an active free radical, by the intervention of an atom or molecule containing an unshared electron, or by separation of an electron pair; (b) reaction of the free radical with oxygen to form a peroxidic radical; (c) interaction of the latter with the substrate to form the oxidation product and regenerate the original free radical.* The chain is broken when two similar radicals react, or when two unlike radicals react to form the addition product. An inhibitor



such as hydroquinone may interrupt the chain through diversion of the peroxidic free radical in a reaction (d) which does not regenerate the free radical of the aldehyde, or by direct diversion of the original free radical. The inhibitor may be regenerated by interaction with the accessory products of the initial activation (e).

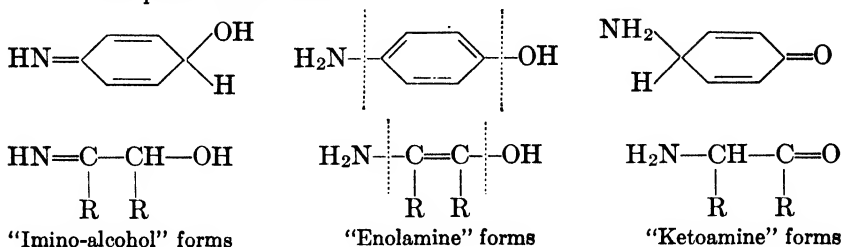
It is significant that aromatic systems, containing hydroxyl and amino substituents in an *ortho* or *para* relationship, are among the most powerful auto-oxidation inhibitors.¹⁰¹ These compounds may be regarded as vinylogs of the parent inorganic structures, HO—OH, NH₂—OH and NH₂—NH₂. In the organic types the discordant sys-

¹⁰⁰ Haber and Willstätter, *Ber.*, **64**, 2844 (1931).

* For detailed mechanisms for chain reactions of this type and modifications of the Haber-Willstätter mechanism, see Wieland and Richter, *Ann.*, **486**, 226 (1931); **495**, 284 (1932); and Rice and Rice, "The Aliphatic Free Radicals," Johns Hopkins Press, Baltimore (1935), pp. 170-181.

¹⁰¹ Moureu and Dufraisse, *Chem. Rev.*, **3**, 113 (1927); Milas, *ibid.* **10**, 296 (1932).

tems can be relieved by tautomerism of a sort that is different from that of the parent structures.

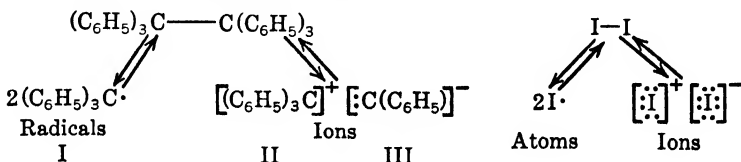


The "enediol" and "enolamine" forms of α -hydroxy and α -amino carbonyl structures may be regarded as vinylogs of the peroxidic systems $\text{HO}-\text{OH}$ and NH_2-OH , and this analogy makes possible an interesting correlation of their behavior in biological reactions. Thus, the relation between an α -amino acid and the corresponding α -keto acid is analogous to that of an aminophenol and the corresponding quinone; the α -hydroxy and α -keto acids become analogous to hydroquinone and quinone, which in turn correspond to $\text{HO}-\text{OH}$ and $\text{O}=\text{O}$.

FREE RADICALS

A free radical is a molecular species, usually electrically neutral, in which is present an atom bearing a single unshared electron. Such structures contain an uneven number of valence electrons and have been designated by Lewis¹ as "odd molecules." Free radicals are therefore an exception to the most fundamental principle of chemical combination ("rule of two"), and they exemplify the highest degree of molecular unsaturation (p. 489).

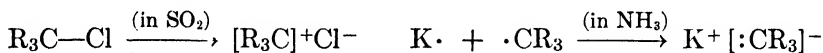
Free radicals resemble free atoms, such as monatomic hydrogen and chlorine, or alkali metals. Although the notion of organic radicals goes back as far as Lavoisier, the first experimental evidence of their real existence was given by Gomberg's discovery¹⁰² of triphenylmethyl in 1900. The parent substance hexaphenylethane is capable of reversible dissociation either into neutral free radicals or into ions, and in this respect bears a strong formal resemblance to molecular iodine.



¹⁰² Gomberg, *J. Am. Chem. Soc.*, **22**, 757 (1900); **36**, 1144 (1914); *Chem. Rev.*, **1**, 91 (1924); **2**, 301 (1925).

Subsequently, a large number of free radicals of the triarylmethyl type have been prepared and also free radicals containing unsaturated atoms of nitrogen, arsenic, oxygen, sulfur, tin, and lead (pp. 526-528).

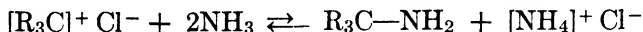
A satisfactory interpretation of the mass of experimental observations on free radicals of the triarylmethyl type requires a recognition of the existence of three definite species: electrically neutral triarylmethyl radicals (I) and triarylmethyl cations (II) and anions (III). The free radical is formed alone in non-ionizing solvents, and the ions in ionizing solvents. Triarylmethyl cations are present alone (probably in a solvated state) in solutions of triarylmethyl halides in liquid sulfur dioxide; the corresponding anions exist alone in solutions of alkali metal salts in liquid ammonia.



The triphenylmethyl anion is dark red and is the most highly colored of the three species; the free radical is yellow, and the cation is either colorless or yellow. Experiments of Wallis and Adams⁹⁵ indicate that an unsymmetrically substituted triarylmethyl anion can exist in an optically active state, but that the corresponding cation undergoes racemization rapidly (p. 322).

The ability of aryl groups to increase the capacity of an attached atom to absorb an electronic deficit or excess (p. 1665) accounts for the effect of aryl groups in stabilizing the ions of opposite sign formed by ionic dissociation. The power of aryl groups to stabilize the neutral radical containing a single unshared electron is due to the same fundamental property—their ability to distribute the singlet to a large number of positions in the system (resonance). This view accounts for the fact that polynuclear aromatic systems are more effective than phenyl in stabilizing the radical, and the theoretical sequence— α -naphthyl > β -naphthyl > *p*-xenyl > phenyl—is in agreement with the known facts. The presence of substituents having a considerable +*I* effect and a -*T* effect (alkoxyl, halogens) should also increase the extent of dissociation, and this is found to be true.

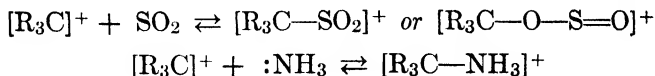
Ingold¹⁰³ holds the view that “the triphenylmethyl anion is obviously much less stable than the kation.” As an illustration of the stability of the cation is cited the observation¹⁰⁴ that triphenylmethyl chloride is soluble in liquid ammonia with only slight, and reversible, conversion into the corresponding amine (ammonolysis):



¹⁰³ Ingold, “Ann. Repts. Chem. Soc. (London),” **25**, 155 (1928).

¹⁰⁴ Kraus and Rosen, *J. Am. Chem. Soc.*, **47**, 2739 (1925).

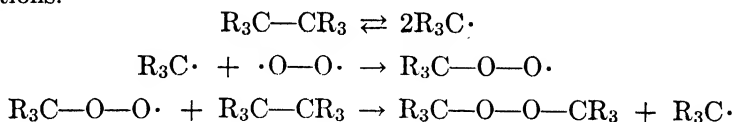
Ingold's view appears to ignore the circumstance that triarylmethyl cations are actually formed only in donor solvents (NH_3 , SO_2) capable of stabilizing the cation by solvation, whereas the triphenylmethyl anion exists as such in solvents and in the solid alkali metal salts.



Evidence that hydrocarbon anions are actually more stable than the corresponding cation is afforded by the fact that in many cases a cation undergoes internal stabilization by intramolecular rearrangement whereas the corresponding anion does not. The relatively greater configurational stability of the triaryl anions, already cited, may be considered to throw further doubt upon the view expressed by Ingold.

Even relatively stable free radicals, such as the triarylmethyls, are extremely reactive substances. They react readily with alkali metals, molecular oxygen, iodine, nitric oxide, and other free radicals; with ethers, esters, ketones, nitriles, and hydrocarbons they form additive compounds involving one molecule of the substrate and two of the free radical. Studies of the velocity of dissociation of hexaphenylethane by the addition of a reagent (halogens, nitric oxide) that reacts instantly with the free radical show that the reaction is strictly unimolecular and is almost independent of the solvent.⁸⁶ The period of half-change of hexaphenylethane was found to be 3.3 minutes.

The behavior of hexaphenylethane toward oxygen¹⁰⁵ was found to involve the formation of a labile peroxide which gives rise to chain reactions.



In the presence of an excess of pyrogallol the reaction follows a strictly unimolecular course, and exactly two molecules of oxygen are consumed per molecule of the ethane. The inhibitor functions by its ability to effect an instantaneous fixation of the labile peroxide, converting it to $\text{R}_3\text{C}-\text{O}-\text{OH}$.

Free alkyl radicals were first prepared and studied by Paneth¹⁰⁶ in 1929. Free methyl and ethyl radicals were obtained by thermal decomposition of the lead alkyls in a stream of pure hydrogen at low

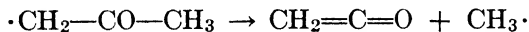
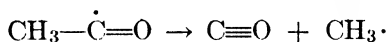
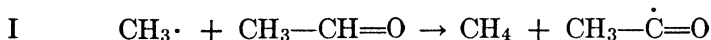
¹⁰⁵ Ziegler and Ewald, *Ann.*, **504**, 162 (1933).

¹⁰⁶ Paneth and Hofeditz, *Ber.*, **62**, 1335 (1929); Paneth and Lautsch, *Ber.*, **64**, 2702 (1931).

pressures (1–2 mm.). The free alkyls were found to effect direct alkylation of such inactive elements as lead, antimony, zinc, cadmium, bismuth, and tellurium. Further work by Rice and his collaborators¹⁰⁷ has shown that free alkyl radicals react readily with alkali metals, calcium, mercury, lanthanum, thallium, arsenic, and selenium; no alkylation was observed with magnesium, copper, silver, gold, and cerium. The products formed by reaction with arsenic, antimony, and bismuth¹⁰⁸ consist of trialkyls, dialkyls of the cacodyl type, and polymeric monoalkyls (except with bismuth). With tellurium only dimethylditelluride, $\text{CH}_3\text{—Te—Te—CH}_3$, is formed and no dimethyltelluride, $(\text{CH}_3)_2\text{Te}$.

The half-life period of the methyl and ethyl radicals is only about 0.006 sec., which is even shorter than that of atomic hydrogen under similar conditions (ca. 0.1 sec. Experimental studies of the higher alkyl radicals * indicates that these decompose readily into methyl or ethyl radicals and olefins. It is estimated that about 75 per cent of the free *n*-butyl radicals formed by the primary thermal decomposition of di-*n*-butylmercury break up into ethylene molecules and ethyl radicals.¹⁰⁷

A consideration of the reactions of free alkyl radicals with organic molecules (in the gaseous state), based upon the assumption of free radicals in thermal and photochemical decomposition, indicates that they attack the carbon-hydrogen link and not the carbon-carbon link.¹⁰⁷ The thermal or photochemical decomposition of acetaldehyde is interpreted from a free radical standpoint by the following chain mechanism (I):



A methyl radical, produced by thermal or photochemical excitation, attacks the C—H link of the aldehyde molecule producing methane and a labile aldehyde radical. The latter decomposes rapidly with loss of carbon monoxide and regenerates a methyl radical, which continues the

¹⁰⁷ Rice and Rice, "The Aliphatic Free Radicals," Johns Hopkins Press, Baltimore (1935).

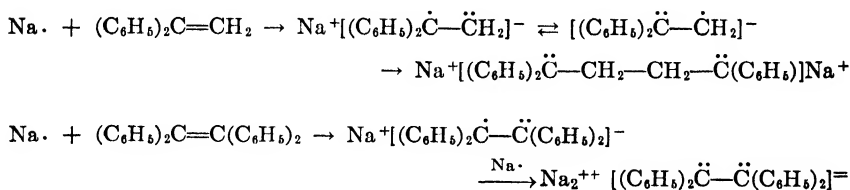
¹⁰⁸ Paneth, *Trans. Faraday Soc.*, **30**, 179 (1934).

* Evidence for the existence of the *n*-propyl radical has been obtained by Pearson and Purcell, *J. Chem. Soc.*, 253 (1936); its half-life period is estimated to be about 0.002 sec., which is only one-third that of the methyl or ethyl radical.

cycle. It is found experimentally that the products are entirely methane and carbon monoxide. The pyrolysis of acetone to yield ketene and methane (II) is explained by a similar mechanism.¹⁰⁷ But the photochemical decomposition of acetone yields ethane and carbon monoxide, and it is difficult to account for this difference if both reactions are assumed to occur by way of free methyl radicals.

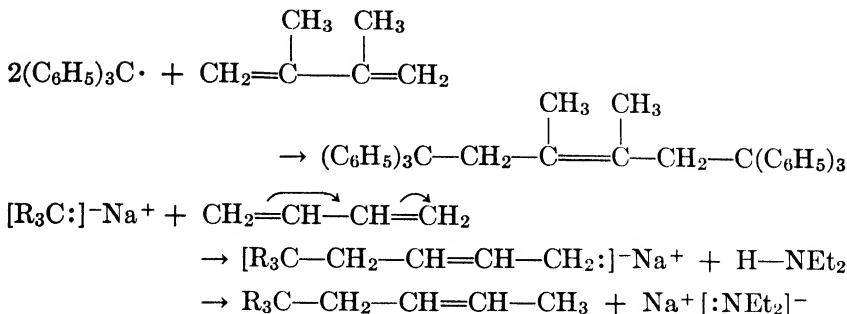
The possibility of free radicals being formed as intermediate products in the course of chemical reactions in the liquid state or in solutions is only occasionally supported by the experimental evidence. In general, ionic or pseudo ionic mechanisms (p. 1631) are the more common modes of reaction, and free radicals arise only under rather special conditions. There is convincing evidence that thermal and photochemical decompositions occur by way of radical chains. Other reactions in which free radicals may arise are those involving alkali metal atoms (metal ketyls, Wurtz-Fittig reaction), monatomic hydrogen, molecular iodine, molecular oxygen, hydrogen peroxide (and other peroxidic systems), quinones, nitric oxide (and odd molecules in general), atoms and ions of the transition elements, and electrolysis.

The addition of metallic sodium or lithium to arylated olefins (p. 422) and dienes may be cited as an illustration of a reaction involving free radicals. Schlenk and Bergmann¹⁰⁹ found that an atom of sodium initially adds a single electron to the carbon directly attached to the aromatic ring, giving a product analogous to the metal ketyls. The



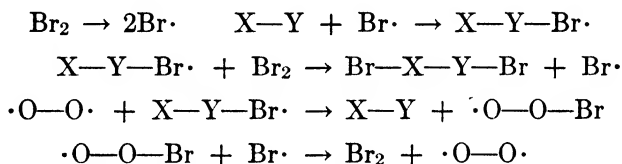
subsequent course of the reaction is determined by the stability of the initial product. In the case of 1,1-diphenylethylene, two of the units combine to give the disodium derivative of 1,1,4,4-tetraphenylbutane. With tetraphenylethylene, dimerization does not occur but reaction with a second atom of sodium gives the disodium derivative of tetraphenylethane. Conant and Scherp⁸⁷ have found that isoprene and 2,3-dimethylbutadiene add two molecules of triphenylmethyl in the 1,4-positions, and this reaction can be formulated in a similar manner; there is also the possibility that it occurs by an ionic mechanism.

¹⁰⁹ Schlenk and Bergmann, *Ann.*, **463**, 1 (1928).



Ziegler and his collaborators¹¹⁰ have found that alkali metal alkyls are active polymerizing agents for 1,3-dienes. By arresting the polymerization with diethylamine, phenylisopropylpotassium and butadiene gave 1-(phenylisopropyl)-butene. The mechanism of this polymerization is not clear, but if the active reagent is the alkyl ion, the process would follow an ionic mechanism. This view finds some support in the observation that the effectiveness of the alkali alkyls decreases in the order—benzyl > phenylisopropyl > triphenylmethyl—which is the reverse of the anionic stabilities and therefore parallels their donor activity.

The observation that molecular oxygen arrests the photocatalyzed addition of bromine to cinnamic acid¹¹¹ suggests that a radical chain occurs in the reaction.



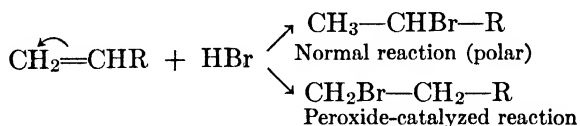
Molecular oxygen may arrest the chain reaction by conversion of the labile bromo-olefin radical (formulated as $\text{X}-\text{Y}-\text{Br}\cdot$) into the original olefin and a stabler bromo-oxygen radical; the latter does not attack the olefin but can react with a bromine atom to regenerate molecular oxygen and bromine. The addition of halogens and halogen acids to olefins in the dark and in the presence of polar catalysts or solvents appears undoubtedly to involve an ionic mechanism (p. 1675), and in these cases molecular oxygen has little or no effect.

In some instances it appears that the olefin itself reacts with molecular oxygen to form a labile peroxide and that the latter may alter the

¹¹⁰ Ziegler and collaborators, *Ann.*, **511**, 13, 45, 64, 101 (1934).

¹¹¹ Daniels and Bauer, *J. Am. Chem. Soc.*, **56**, 2014 (1934).

mechanism of addition so as to bring about a reversal of the orientation of addition (p. 549). Studies by Kharasch and his collaborators⁷⁰ indicate that the addition of hydrogen bromide to highly purified olefins in the absence of molecular oxygen or peroxides (or in the presence of anti-oxygens) occurs in the direction anticipated from theoretical considerations. This reaction, designated as the normal addition, follows a polar mechanism and usually occurs much less rapidly than the



peroxide-catalyzed reaction. It is possible that the peroxide-catalyzed addition occurs through an intermediate radical, but the detailed mechanism is uncertain. The orientation of addition of hydrogen iodide to olefins is not reversed in the presence of peroxides, but this result may be due to the destruction by hydrogen iodide of the original peroxide or of a labile intermediate.

TAUTOMERISM

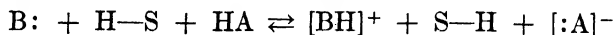
Tautomeric structures were classified by Laar¹¹² in 1885 as dyad, triad, tetrad, and pentad systems, depending upon the number of atoms intervening between the initial and final positions of the mobile hydrogen atom. This classification serves as a convenient basis for consideration of their electronic characteristics.¹¹³ The ionic mechanism of tautomeric change (ionotropy) is now clearly established, and the two different types of exchange are distinguished by the terms prototropy and anionotropy. The salient features of prototropic change, without reference to the actual mechanism, are the following: (a) separation of a proton, (b) redistribution of the disengaged electron-pair by electro-meric displacements in the resulting anion, (c) recombination of a proton at the new anionic center. Anionotropic change has the converse ionic relationship, involving separation of an anion and redistribution of the resulting electronic deficit (open sextet).

As a matter of convenience tautomeric change is formulated frequently as an intramolecular migration, but physical evidence indicates that the process involves an actual separation of the ions concerned. Prototropic change does not take place in the vapor or in the solid state;

¹¹² Laar, *Ber.*, **18**, 648 (1885).

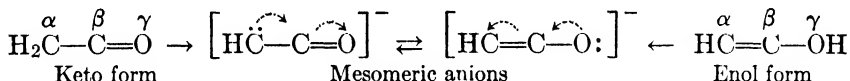
¹¹³ Baker, "Tautomerism," Routledge and Sons, London (1934); see, also, Waters, "Physical Aspects of Organic Chemistry," Routledge and Sons, London (1935).

it is catalyzed by proton-donors and proton-acceptors (acids and bases), and is facilitated by an amphoteric solvent such as water. Brönsted and Guggenheim¹¹⁴ have formulated the isomeric change in the presence of an amphoteric solvent by the general equation:



The symbols H—S and S—H are used to indicate the two forms of a prototropic compound; B and HA represent a basic catalyst and its conjugate acid. It will be observed that either isomer should give rise to an identical anion; an essential feature of the tautomeric relationship is a condition of equilibrium between two or more resonating structures (unperturbed states) of the anion.

The condition of resonance may be indicated by writing the structure of one of the final (unperturbed) states and attaching the usual curved arrow symbols showing how the electronic configuration should be modified to represent the actual electronic distribution in the system.* The keto-enol systems provide typical illustrations. The conversion of one form of the anion into the other does not require the rearrange-



ment of any atomic nuclei, and the anion may react chemically as if it possessed either structure. As the terminal atoms are not identical, the distribution of the anionic charge between α and γ is unequal and will be determined by their relative electron-attraction (effective nuclear charges) and polarizabilities, including the influence of attached groups (internal factors) and of the environment (external factors).

Dyad Systems. Lapworth¹¹⁵ pointed out many years ago that tautomerism in odd-numbered systems (triad and pentad) does not alter the valence of any atom, but when an even number of atoms is involved (dyad and tetrad) the valence of one of the terminal atoms is changed by two units. Consequently, dyad and tetrad tautomerism can arise only in systems containing a terminal atom of variable valence, such as

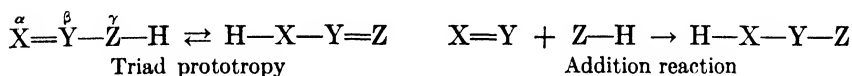
¹¹⁴ Brönsted and Guggenheim, *J. Am. Chem. Soc.*, **49**, 2554 (1927).

* Ingold (*J. Chem. Soc.*, 1120 [1930]; *Chem. Rev.*, **15**, 225 [1934]) has pointed out that this notation does not distinguish between an electromeric polarizability effect and a mesomeric polarization, for the curved arrows merely denote a mechanism of electronic displacement which is supposed to characterize a molecular state or a process occurring in the course of a reaction. He has introduced curved bond signs without arrows to indicate the "distributed" electron pairs: $\text{O}-\text{CR}-\text{O}$ would represent the mesomeric state of a carboxylate anion rather than $\text{O}=\text{CR}-\text{O}$.

¹¹⁵ Lapworth, *J. Chem. Soc.*, **73**, 457 (1898)

the "tautomeric" forms lose their structural identity. Thus, the distinction between an oxime and a nitron vanishes when dimerization occurs. Similar resonating forms may be produced also in certain triad prototropic systems, especially those in which the terminal atoms are oxygen and nitrogen (amides, amidines, diazoamino compounds, etc.). It is remarkable that these are the particular cases in which all attempts to separate the individual tautomeric forms have been unsuccessful.

Triad Systems. The best-known examples of tautomerism are the prototropic triad and pentad systems. These may be represented by a general equation, in which the atoms X, Y and Z may be carbon, nitrogen, or oxygen.



A close relationship exists between tautomerism and reversible addition reactions;¹¹⁶ the mechanism of tautomeric change is an intermolecular process involving proton addition and elimination (p. 1705). The more important types of triad systems are shown in Table XVIII. Pentad systems may be regarded as extensions (vinylogs) of the corresponding triad structures.

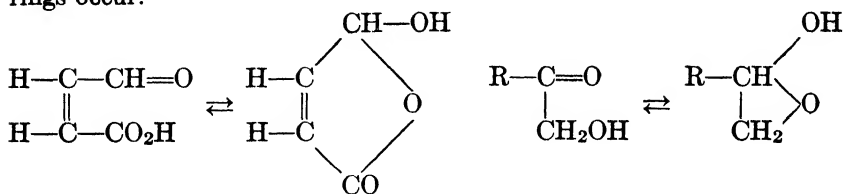
TABLE XVIII
TRIAD TAUTOMERIC SYSTEMS

Three carbon.....	H—C—C=C	C=C—C—H
Keto-enol.....	H—C—C=O	C=C—O—H
Imino-enamine.....	H—C—C=N	C=C—N—H
Nitrile-imine.....	H—C—C≡N	C=C=N—H
Azo-hydrazone.....	H—C—N=N	C=N—N—H
Nitroso-oxime.....	H—C—N=O	C=N—O—H
Aci-nitro.....	H—C—N=O	C=N—O—H
	↓ O	↓ O
Amide-imidol.....	H—N—C=O	N=C—O—H
Amidine.....	H—N—C=N	N=C—N—H
Diazoamino.....	H—N—N=N	N=N—N—H
Nitrosoamino.....	H—N—N=O	N=N—O—H
Allylic.....	X—C—C=C	C=C—C—X

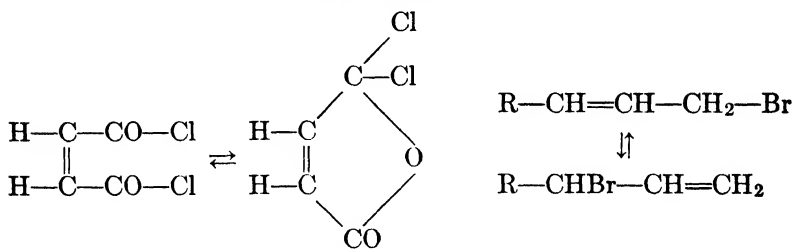
Ring-chain tautomerism affords a striking illustration of the analogy between tautomeric change and reversible additive reactions, since the ring form is an obvious addition product. The rings most frequently encountered are five-membered cycles containing one double bond and

¹¹⁶ Ingold, *ibid.*, **123**, 1706 (1923).

five- or six-membered saturated cycles; occasionally, three-membered rings occur.

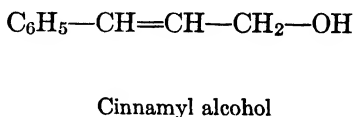
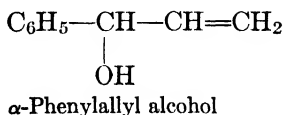


Ring-chain tautomerism



Anionotropic systems

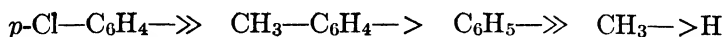
The recognition of anionotropic triad systems is comparatively recent,⁷⁹ and the examples are limited almost entirely to the three-carbon (allylic) type. Interconversion of the 1,2- and 1,4-adducts of conjugated dienes, the *sym.*- and *unsym.*-phthalyl chlorides, and derivatives of cinnamyl and phenylallyl alcohols¹¹⁷ are typical examples. The study of anionotropic change has not yet advanced as far as that of prototropic change, but it is of interest to note that a number of generalizations relating to their mobility and equilibrium, deduced from theoretical considerations, have been verified experimentally. The tendency to migration in the α -phenylallyl—cinnamyl series for different potential anions follows the same sequence as the ionic stability: bromide > acetate > alcohol.



The individual alcohols can be obtained separately, and each can be esterified without a change of structure. Conversion of the α -phenylallyl esters to the cinnamyl esters can be effected by heating in a solvent; the rate of conversion varies with the ionizing power (dielectric constant) of the solvent: benzonitrile, acetic anhydride > chlorobenzene

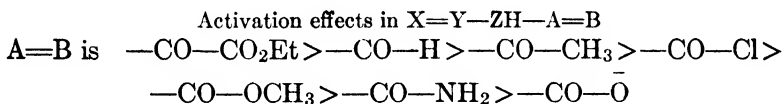
¹¹⁷ Burton and Ingold, *ibid.*, 904 (1928); Burton, *ibid.*, 248 (1930).

> *p*-xylene. Isomerization of α -phenylallyl bromide is extremely rapid, so that the alcohol yields cinnamyl bromide when treated with hydrobromic and acetic acids. The observed influence of α -substituents upon the mobility of allylic systems is in agreement with the anticipated



sequence,⁹⁸ based upon the view that the mobility is increased by any group which can facilitate electron release ($-I$ or $-T$ effect).

The most effective activating groups for prototropic change will be those that have a strong electron attraction *and* can also provide a suitable seat for the charge on the electromeric anion. An ammonium group $-\text{NR}_3^+$, in spite of its powerful electron-attraction ($+I$), does not satisfy the second requirement and, consequently, has only a weak activating influence. On the other hand, nitrile and carbonyl groups, which satisfy both requirements, have an extremely powerful activating effect. The relative activating effects in a series of carbonyl structures $-\text{CO}-\text{R}$ will be enhanced by the ability of R to reinforce the electron-attraction of the carbonyl carbon ($+I$), but will be diminished by an ability of R to furnish electrons by electromeric electron-release (see Neutralized Systems, p. 1680). On this basis the activating influence of a series of groups, substituted at the α - or γ -position of a triad system, would decrease in the following order:



The anticipated order is in excellent agreement with experimental observations of the behavior of prototropic systems. It is of interest to note that substitution at the β -position has much less effect than at the α - or γ -positions. Indeed, if the terminal atoms X and Z in a triad system $\text{X}=\text{Y}-\text{ZH}$ remain constant, variations of Y have but little influence on the mobility of the system.

Pentad Systems. When two activating groups $\text{X}=\text{Y}$ and $\text{A}=\text{B}$ are attached to the same atom, there results a pentad system of the general form $\text{X}=\text{Y}-\text{ZH}-\text{A}=\text{B}$. Many of the best known examples of tautomerism fall into this class, which may be regarded as "extended" or "double" triad systems. In the pentad structures the mobility is determined largely by the characteristics of X, Z, and B; the atoms Y and A are much less significant.

In simple keto-enol triads the position of equilibrium is very strongly toward the keto form, and effective enolization is usually brought about

through the influence of powerful reagents (strong acids or bases). In general the extent of enolization is greater in the pentad systems, and in many cases the equilibrium mixture contains more than 50% of the enol. The large amount of enol is probably due to the circumstance that the pentad systems permit the formation of chelate structures involving 2-covalent hydrogen (p. 1638) which derive additional stability from resonance effects. The phenols afford an excellent example of a parallel phenomenon; the enol form of a phenol is stabilized through the participation of the $C=C$ in the resonance of the aromatic nucleus.

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A survey of the applications of modern electronic theories of chemical reaction reveals that much progress has been made in the direction of correlating the vast subject matter of organic chemistry. The modern theories are more definite in a physical sense and yet are broader in aspect than the former theories. It is evident, of course, that many of the individual postulates and general ideas of the modern theory had existed in the earlier conceptions of Kekulé, Michael, Thiele, Lapworth, Flürscheim, Noyes, Stieglitz, and others. The new theories appear to present the essential truths of the older views in a more precise and unambiguous fashion, to eliminate misconceptions and inconsistencies in the older views, and to bring together many apparently isolated phenomena.

An important contribution of the modern electronic concepts of valence as a basis for the interpretation of reaction mechanisms is this: the imposition, by the introduction of a few fundamental generalizations (especially the principle requiring the maintenance of stable electronic configurations), of certain definite limitations upon the forms of electron displacement which it is permissible to assume in the course of chemical change.

In conclusion it is appropriate to note briefly a few of the significant contributions of the modern theories. The recognition of two kinds of valence forces, electrovalence and covalence, has led to more accurate molecular models of organic systems and has rectified errors in the older structural formulas. The broad concept of electronic resonance (mesomerism) has been of great value in correlating structure and chemical reactivity. The notion that a hydrogen atom can hold two atoms together (2-covalent hydrogen, or hydrogen-bridge) has served to bring together and clarify a large number of experimental observations that had long been regarded as unique or unrelated phenomena.

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CHAPTER 20

CONSTITUTION AND PHYSICAL PROPERTIES OF ORGANIC COMPOUNDS

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INTRODUCTION

Any definition of a physical as distinguished from a chemical property is necessarily arbitrary since it is the physical properties of atomic dimensions and interatomic forces which determine both the physical and chemical behavior of molecules. Certain properties such as boiling point, refractive index, dipole moment, and many others have been commonly termed physical; other properties such as valence and thermodynamic functions have been called both chemical and physical. For the purposes of this chapter no definition of a physical property will be given, the commonly understood meaning of the term being accepted and extended to include such borderline properties as free energy and heat of combustion.

The objectives of the systematic study of the physical properties of organic compounds may be arbitrarily divided into four types. One of these objectives is the direct determination of molecular structures and of interatomic forces; another is the determination of molecular structures by analogy, i.e., by comparing certain properties of compounds of unknown structure with those of compounds whose structures are known. A third objective is the prediction of the course and extent of chemical reactions. A fourth purpose of the study of physical properties is to provide a method for predicting the properties of unknown compounds, or of known compounds whose properties have not been measured, and to afford a means of checking the accuracy of the physical constants of compounds.

It is generally true that the study of a given property will be useful in connection with only one of the four objectives given, but it sometimes happens that the simultaneous study of several properties leads to results which are of value for more than one purpose. For example, the systematic study of densities enables the prediction of unknown densities to be made, and studies of surface tension are useful for a similar reason; when these two properties are simultaneously measured for the same compounds, the results can be combined to yield parachor constants which may be used in determining molecular structures by analogy.

The value of the study of a given physical property depends to a considerable extent upon the care with which the study was designed and upon the accuracy with which the measurements were made. Lack of appreciation of these two factors by the experimenters has destroyed the utility of many such studies which are reported in the literature, and caution must be exercised in evaluating many of the published data. Although a large percentage of the older data must be discarded, some of

these data are quite trustworthy and have not been superseded up to the present time.

The measurement of certain physical properties of molecules leads directly to a knowledge of the actual arrangement of the atoms in the molecules and of the forces which hold the atoms together. The study of x-ray and electron diffraction patterns, of absorption spectra, and of the Raman effect has been of particular value in giving a definite understanding of the molecular configuration of simple molecules or of those exhibiting high degrees of symmetry. The analysis of data on dielectric constants leading to values of dipole moments has been very useful in affording knowledge of the electrical symmetry of molecules.

The theoretical importance of determining molecular configurations and interatomic forces is obvious. From the point of view of more immediately practical use, such information is becoming increasingly valuable because of recent advances in theoretical and experimental studies of reaction kinetics; it is probable that in the future it will be possible to calculate the rates of reaction (p. 802) of molecules in homogeneous systems from such data. It has already proved convenient and reliable to calculate the equilibrium constants of certain reactions involving simple molecules from data obtained by studies of their absorption and Raman spectra.

The study of another physical concept, thermodynamics, is important for other reasons. Thermodynamics tells us what reactions will proceed and how far they will go although it gives no information about the mechanisms of the reactions nor about the microscopic states of molecules. The development of a body of thermodynamic data on organic compounds is a slow and laborious procedure, but the existence of such data will have great practical value.

By far the majority of the published data on physical properties gives no insight into molecular structure and does not allow the prediction of the course or extent of chemical reactions. The proper interpretation of the vast amount of available information of this sort has its principal value in the prediction of the properties of unknown compounds, in providing a means of identification of materials, and, when applied with caution, in serving as an empirical tool for proving the structures of new compounds by analogy. Of the properties of this type, the most commonly determined and used are density, molecular volume, surface tension, viscosity, melting point, boiling point, and refractive index. A great deal of effort has been devoted and is being devoted at the present time to making accurate determinations of the numerical quantities associated with this group of properties, and these properties will be treated first in this chapter. After a general discussion of the

group as a whole, the individual properties will be considered to lengths consistent with their importance and with the scope and extent of this chapter. The treatment of those properties whose analyses are aimed at the determination of intramolecular configurations and forces will be deferred until the latter part of the chapter.

Since it is obviously impossible to discuss in detail the great amount of material in the literature which is pertinent to the subject, it has been necessary to limit the treatment to the consideration of those properties which are of greatest importance to the organic chemist. Methods of measurement cannot be given here, nor can discussions as to the reliability of data be entered into. For more detailed information the reader should consult in addition to standard texts¹ the more comprehensive treatises to be found on the subject.^{2, 3, 4, 5, 6, 7, 8}

PROPERTIES WHOSE ANALYSES DO NOT LEAD DIRECTLY TO STRUCTURE DETERMINATION

The physical properties belonging to the group which comprises density, molecular volume, surface tension, refractivity, and the like are determined by the kind and number of atoms in the molecules, by the position of the atoms in the molecules, and, except for very dilute gases, by the attractive or repulsive forces which the molecules exert upon each other. Extreme cases in which only one of these factors determines the value of the property being investigated can be found; for example, the molecular weight of a compound is calculated only from the number of atoms in the molecule, the sign of rotation of the plane of polarized light of two stereoisomers is governed solely by the spatial arrangement of the atoms, and the melting point of a pure compound depends almost

¹ Getman and Daniels, "Outlines of Theoretical Chemistry," John Wiley & Sons, New York (1931); Rodebush, "Introductory Course in Physical Chemistry," Van Nostrand Co., New York (1932); Taylor, "A Treatise on Physical Chemistry," Van Nostrand Co., New York (1931), 2 vols.; Eucken, "Grundriss der physikalischen Chemie," 4th ed., Akad. Verlag., Leipzig (1934); Henrich, "Theories of Organic Chemistry," John Wiley & Sons, New York (1922).

² Hückel, "Theoretische Grundlagen der organischen Chemie," Akad. Verlag., Leipzig (1931), 2 vols.

³ Houben and Weyl, "Die Methoden der organischen Chemie," Thieme, Leipzig (1925-7), 4 vols.

⁴ Smiles, "The Relation between Chemical Constitution and Physical Properties," Longmans, Green and Co., London (1910).

⁵ Cohen, "Organic Chemistry for Advanced Students," Arnold & Co., London (1928), 3 vols.

⁶ Freudenberg, "Stereochemie," Deuticke, Leipzig and Wien (1933), 3 vols.

⁷ Glasstone, "Recent Advances in Physical Chemistry," 2nd. ed., Blakiston's Son and Co., Philadelphia (1933).

⁸ "Handbuch der Physik," Springer, Berlin (1926-34), 24 vols.

entirely upon intermolecular forces. The above are exceptional cases; nearly always the numerical value of a property is determined by a combination of all three factors.

For purposes of analysis the detailed consideration of intermolecular forces may be eliminated. This is permissible because such forces are determined by the numbers and arrangements of atoms within molecules and hence are not independent of the latter. In the discussion of such properties as surface tension and viscosity, mention will necessarily be made of intermolecular forces; it must be remembered, however, that these forces bear the same relation to the intramolecular distribution of the atoms as effect to cause.

The various properties of substances have been arbitrarily divided into three classes: colligative, additive, and constitutive. Colligative properties are defined as those which depend solely on molecular concentration; they are of interest only in connection with mixtures of substances and will not be considered here.

The Concept of Additive and Constitutive Properties

Additive properties are those which are influenced by the numbers of atoms in the molecules, e.g., molecular weights. Constitutive properties are those which depend only on the relative spatial configuration of the atoms in the molecule. Almost all properties exhibit both additive and constitutive components which it is difficult or impossible to separate.

The concept of additive and constitutive properties is exceedingly valuable in the analysis of data which are to be used in deriving molecular structures by analogy. The physical constants of a series of compounds are examined and an attempt is made to determine the contributions of the individual atoms or groups of atoms in the molecule by assigning certain numerical values to these atoms or groups, these values being chosen in such a manner that their sum is equal to the total values observed experimentally. Almost always it is found impossible to arrive at satisfactory unit or group values. It is therefore necessary to introduce certain corrections which are determined by the structural characteristics of the molecule. It is the success with which these constitutive corrections are applied that determines the value of the analysis and the utility of the method for predicting properties and verifying postulated structural formulas.

Examples of the Effect of Constitution on Properties

The great importance of the constitutive factor in many physical properties is illustrated by a table of some properties of the isomeric

xylenes. T. W. Richards⁹ has pointed out certain relationships existing between various properties. Thus, additive factors being equal, substances with high boiling points have relatively smaller specific volumes, greater surface tensions, smaller compressibilities, smaller coefficients of expansion, greater molal heats of vaporization, and greater refractive indices than substances with lower boiling points.

TABLE I
COMPARISON OF PHYSICAL PROPERTIES OF ISOMERS

Property	<i>o</i> -Xylene	<i>m</i> -Xylene	<i>p</i> -Xylene
Boiling point.....	144°	139°	136°
Melting point.....	-28°	-54°	+15°
Density.....	0.8811	0.8658	0.8611
Compressibility.....	61.2	64.8	67.5
Coefficient of expansion.....	973	994	1009
Molecular heat of vaporization.....	8.75	8.71	8.60
Surface tension.....	3.09	2.96	2.92
Refractive index.....	1.5078	1.5002	1.4985
Pressure to produce M.P. at 30°.....	490	2400	4000

Studies by Schurman and Boord¹⁰ on the effect of the shift of the double bond on the properties of a number of isomeric olefins afford other examples of constitutive influences. In Table II are collected data on the mono-olefinic derivatives of *n*-octane; the properties of *n*-octane are included as reference points. It is seen that except for 1-octene the boil-

TABLE II
DERIVATIVES OF *n*-OCTANE

Structure	Boiling Point (760 mm.)	Density, d_4^{20}	Index of Refraction, n_D^{20}
C—C—C—C—C—C—C—C	125.59	0.70279	1.39760
C—C=C—C—C—C—C—C	124.6–124.9	.7193	1.4147
C—C—C=C—C—C—C—C	122.7–122.9	.7181	1.4139
C—C—C—C=C—C—C—C	121.9–122.3	.7184	1.4140
C=C—C—C—C—C—C—C	120.9–121.2	.7151	1.4091

⁹ Richards, *Trans. Faraday Soc.*, **24**, 111 (1928).

¹⁰ Schurman and Boord, *J. Am. Chem. Soc.*, **55**, 4930 (1933); this paper contains references to earlier work in this series.

ing points become increasingly lower than that of *n*-octane as the double bond approaches the center of the molecule. The refractive indices and densities do not vary regularly with the position of the unsaturated linkage.

Table III presents data from which may be seen the effect of branched chains and the position of the double bond on the properties of hexenes. In the table, Δ values represent deviations from the properties of the corresponding saturated hydrocarbons.

TABLE III
HEXENES (TYPE I)

Structure	Δ B.P.	Δd_4^{20}	Δn_D^{20}
$\begin{array}{c} \text{C} \\ \\ \text{C}=\text{C}-\text{C}-\text{C} \\ \\ \text{C} \end{array}$	-9.5	+ .0011	+ .0050
$\begin{array}{c} \text{C} \\ \\ \text{C}=\text{C}-\text{C}-\text{C}-\text{C} \\ \\ \text{C} \end{array}$	-9.5	+ .0054	+ .0042
$\begin{array}{c} \text{C} \\ \\ \text{C}=\text{C}-\text{C}-\text{C}-\text{C} \\ \\ \text{C} \end{array}$	-6.5	+ .0138	+ .0081
$\text{C}=\text{C}-\text{C}-\text{C}-\text{C}-\text{C}$	-5.1	+ .0137	+ .0107

Other general studies by Midgley and Henne,¹¹ by van Arkel,¹² by Vogel,¹³ and by Calingaert and Hladky¹⁴ on the relation between constitutive factors and a variety of physical properties of hydrocarbons, esters, acids, and other compounds have recently appeared.

Discussion of Specific Properties

In the following sections will be found a few of the assigned individual group and linkage values for some of the physical properties which have been empirically analyzed with some degree of success. Included with these values are the constitutive correction factors for double bonds, triple bonds, primary, secondary, and tertiary groups, conjugated groupings, cyclic systems, etc. In most of the studies of physical properties it has been necessary to remove certain variable factors by main-

¹¹ Midgley and Henne, *Ind. Eng. Chem.*, **22**, 542 (1930).

¹² van Arkel, *Rec. trav. chim.*, **53**, 247 (1934).

¹³ Vogel, *J. Chem. Soc.*, 333 (1934).

¹⁴ Calingaert and Hladky, *J. Am. Chem. Soc.*, **58**, 153 (1936).

taining conditions of observation at a constant or uniform state, i.e., molal volume at constant temperature, specific heat at the critical temperature, etc. As often as possible, the conditions have been chosen to minimize or remove the influence of another property upon the one being measured.

Melting Points and Boiling Points. Perhaps no two properties are more generally measured by the organic chemist than the melting points of solids and the boiling points of liquids. Melting points of solids are invariably given in the literature as fundamental constants for purposes of identification; boiling points are given as less satisfactory indices of the identity of liquids. Although the melting point of a pure solid is usually a reliable means of identification, the boiling point of a pure liquid is far less satisfactory for this purpose unless great care is taken in controlling the conditions of measurement. Aside from the difficulty of determining boiling points accurately, the latter are also less dependent upon traces of impurities than melting points.

Attempts to correlate the structures of compounds with their melting points and their boiling points have enjoyed only limited success. It is difficult to separate the observed data into their constitutive and additive components because these components manifest themselves through intermolecular forces which are only vaguely understood.

Certain empirical relationships have been discovered which are of interest because of their value in prediction and identification. King and Garner¹⁵ observed a definite relation between the number of carbon atoms in a fatty acid molecule and the entropy change on crystallization. They found that in acids containing more than twelve carbon atoms the heat of crystallization, Q , increases at the rate of 2.06 cal. per g.-mol. for every two CH_2 groups added. In the same series the melting points first drop to a minimum at four or five carbon atoms and then rise and gradually become linear. In this, as in most other aliphatic series, the odd and even carbon compounds form two separate series whose melting points eventually approach a common curve.¹⁶ Fig. 1 illustrates the data of King and Garner.

Timmermans¹⁷ has discussed the phenomenon of alternation of melting points of the even and odd members of substituted or unsubstituted paraffin series. He defines normal alternation as that in which the melting-point values of the even-numbered carbon compounds are higher than those of the adjacent odd members of the homologous series. The

¹⁵ King and Garner, *J. Chem. Soc.*, 578 (1931).

¹⁶ (a) Hildebrand and Wachter, *J. Am. Chem. Soc.*, **51**, 2487 (1929). (b) Chuit and Hauser, *Helv. Chim. Acta*, **12**, 850 (1929). (c) Ruzicka, *Bull. soc. chim. Belg.*, **41**, 565 (1932).

¹⁷ Timmermans, *Bull. soc. chim. Belg.*, **38**, 295 (1929).

assignment of a compound to the even or to the odd series is determined by the number of carbon atoms in the normal paraffin chain. Thus, hexanol with six carbon atoms in the chain is a member of the even series, whereas its isomer, 3-methyl-1-pentanol, with five carbon atoms in its principal chain, is a member of the odd series. There is occasional difficulty in definitely assigning odd or even properties to the first mem-

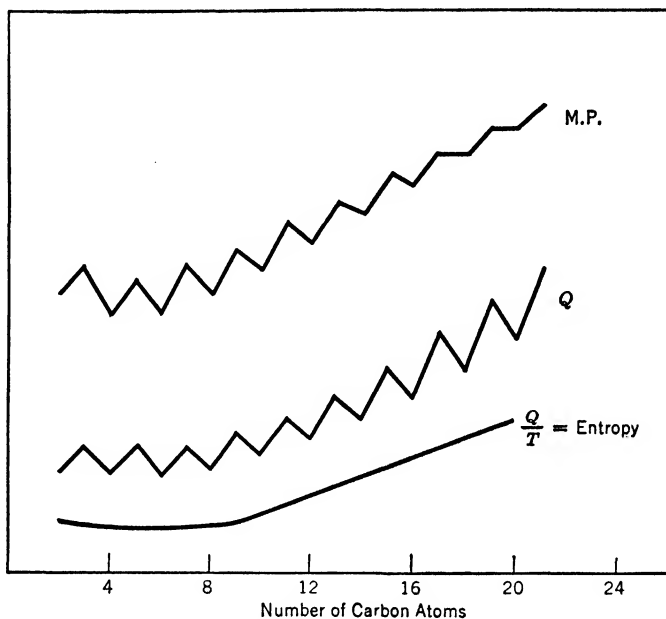


FIG. 1.*—Melting points and heats of crystallization of *n*-fatty acids.
(*Q* = heat of crystallization in cal. per mole.)

ber of an homologous series; for example, toluene and not benzene should be considered the first member of the mono-alkyl benzene series.

It has been observed¹⁸ that the viscosity values of the members of certain homologous series show the same alteration effect as their melting points (Fig. 2). This may indicate that the more viscous compounds are subject to intermolecular forces and orientation effects similar to those existing in crystalline materials.

For the higher members of an homologous series, empirical formulas have been developed from which the melting points can be calculated with reasonable accuracy. Austin¹⁹ has suggested the equation,

$$\log M = A + BT_m$$

* From data of King and Garner, *loc. cit.* (Courtesy of publishers.)

¹⁸ Ceder, *Ann. Univ. Fennicae Alveris, Ser. A2, No. 4* [*C. A.*, **23**, 3137 (1928)].

¹⁹ Austin, *J. Am. Chem. Soc.*, **52**, 1049 (1930).

where M is the molecular weight, T_m is the melting point, and A and B are constants. The constants A and B are generally different for each

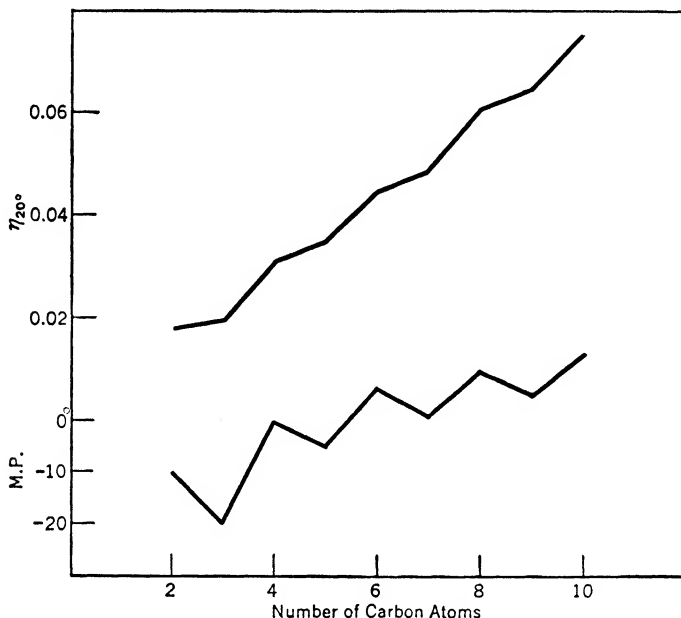


FIG. 2.*—Viscosity and melting points of diethyl esters of dibasic acids.

homologous series (Fig. 3), although the constant B , which gives the slope of the curve, may be the same for several different series. Thus, for n -hydrocarbons and n -alcohols B has the value 0.0040, and hence the $\log M$, T_m curves for these series have the same slope. Variations in the structures of homologous series usually produce changes in the slope of the curves. The curves for monosaccharides, aromatic alcohols, and aromatic acids have negative slopes as contrasted with the positive value of B found for paraffin hydrocarbons. Since constitutive effects are important in

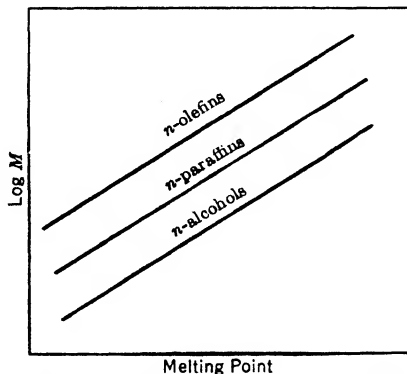


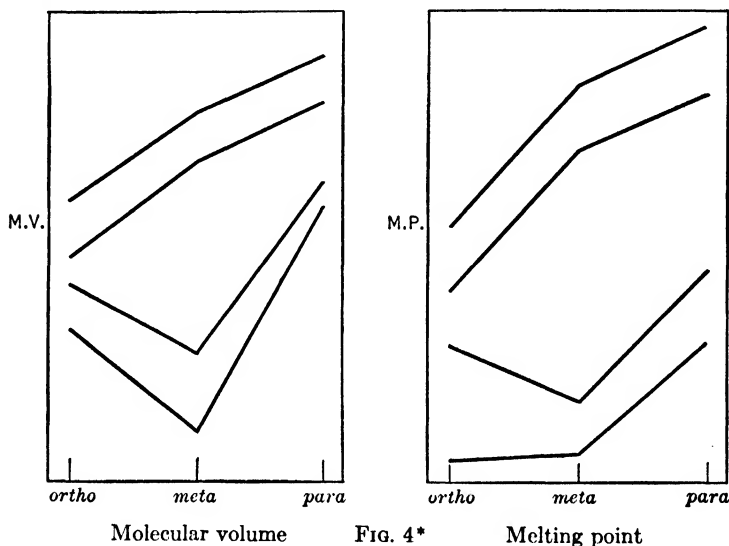
FIG. 3.†—Relation of molecular weight to melting point for homologs.

* From data of Ceder, *loc. cit.* (Courtesy of publishers.)

† Taken from Austin, *loc. cit.* (Courtesy of publishers.)

determining melting points, isomers will not fit on a single curve. In the analysis of the data by Austin no attempt was made to account for the alternating effect of the odd and even carbon compounds; in the higher members of an homologous series this effect often becomes negligible.²⁰

The value of the treatment just discussed lies in its use in predicting the melting points of unknown compounds or in checking the constants of new products. If the melting points of three or four members of an homologous series are known, a curve can be constructed from which,



by extrapolation or interpolation, the melting point of an unknown member of the series can be determined.

Several generalizations can be made. With simple compounds the melting point is higher for molecules exhibiting high symmetry than for those which are unsymmetrical. Of a group of isomers, those having the higher electric moments usually have the lower melting points; for example, *cis* compounds have lower melting points and higher electric moments than *trans* compounds,²¹ while in the aromatic series *ortho* isomers usually have lower melting points and higher moments than *para* isomers. The variation of melting points in a group of isomers finds parallel behavior in changes of other properties²² (Fig. 4).

²⁰ Hildebrand and Wachter, *J. Am. Chem. Soc.*, **51**, 2487 (1929).

* Taken from Klemm and Klemm, *loc. cit.* (Courtesy of publishers.)

²¹ Freudenberg, "Stereochemie," Deuticke, Leipzig and Wein (1933), 3 vols.

²² Klemm and Klemm, *Z. physik. Chem.*, **A151**, 71 (1930).

Boiling points are generally less subject to structural influences than melting points, and correlations between structures and boiling points are not satisfactory. The effect of external pressure on the boiling points of liquids is well known. It has been observed that the members of most homologous series have approximately the same heats of vaporization, i.e., the relationship between the logarithm of the vapor pressure and the absolute temperature is about the same for all members of the series. This observation is of value in predicting the boiling points of compounds at different pressures.

Of the numerous equations developed for the prediction of boiling points, the following expression of Nekrasov²³ may be cited as an example:

$$T_b = K \frac{M - \Sigma}{\sqrt{\Sigma}}$$

In the equation, T_b is the boiling point in degrees absolute, M the molecular weight, Σ the sum of certain empirical equivalents (see Table IV), and K a constant (about 29.0 for hydrocarbons).

TABLE IV
EQUIVALENTS FOR CALCULATION OF THE BOILING POINTS OF ORGANIC COMPOUNDS (Nekrasov)

C	2.0	Each C atom more than 10	+0.25		
H	1.0	=CH ₂	+0.25		
Tert. C in ring	1.50	—CH ₃ on tert. C	+0.25		
Quat. C in ring	1.75	—CH ₃ on quat. C	+0.50		
Double bond in		{=C—C=	-0.50		
2 rings	1.00		{=C=	-1.75	
Benzene ring	1.00	—CH=CH—	-0.75		
Other rings	Sat.	Unsat.	Sat.	Unsat.	
3 members	+0.75	5 members	0.00	+0.50
4 members	+0.20	+1.00	6 members	0.00	0.00

The calculation of the boiling point of ethane will serve as an example of the use of the data given in Table IV. Ethane has 2 carbon atoms and 6 hydrogen atoms; the value of Σ is equal to $2 \times 2 + 6 \times 1 = 10$. Using the value 29.0 for K , T_b is calculated to be 183° K. The boiling point of ethane is observed to be 185° K.

Nekrasov²⁴ has pointed out that the formula $T_b = \frac{KM^{3/4}}{\Sigma}$ is applic-

²³ Nekrasov, *ibid.*, A141, 378 (1929).

²⁴ Nekrasov, *ibid.*, A148, 216 (1930).

able when the compound whose boiling point is being calculated is homopolar in character.

Values more accurate than these derived by the use of Nekrasov's simple equation can be computed by considering in more detail the various structural influences in the molecule. The necessity of applying a great number of constitutive factors in calculating boiling points argues that the relation between structure and boiling point is not understood. In some series of calculations as many as 250 values for special types of linkages and groups are employed, and the necessity for such a number of individual factors detracts from the utility of the method. Nevertheless, the boiling points of some 1500 compounds have been fairly accurately predicted. For example, the boiling points of the isomeric hydrocarbons, sabinene, and pinene, are given in the literature as 438 and 429° K., respectively; they are calculated to be 438 and 428° K.

Other formulas have been proposed,^{25, 26, 27} but will not be discussed here.

Van Arkel²⁸ in a series of papers on the relation between boiling points and dipole moments of isomeric olefins has offered the following generalizations:

1. A 1,2-dialkyl substituted ethylene has a higher boiling point than the mono substituted ethylene of the same molecular weight.
2. A 1,2-dialkyl substituted ethylene has a higher boiling point than the corresponding 1,1-compound.
3. The replacement of a *n*-alkyl group in an olefin by an *iso* group results in the lowering of the boiling point by about 10° C.; the replacement of the *iso* group by a tertiary group causes an additional 10° C. decrease.

Internal Pressure, Association, Solubility, and Surface Tension.

In many of the properties of liquids, the effect of constitutive and additive factors is made apparent only indirectly through their effect on intermolecular forces. The properties of internal pressure, association, solubility, and surface tension fall into this category since each depends directly on the attractive forces exerted between the molecules of the liquid. Although it is not too difficult to obtain at least a relative measure of the intermolecular forces exerted by molecules in the gaseous state, no completely satisfactory method has been devised for making measurements of these forces in liquids. As a result, the interpretations

²⁵ Billig, *Svensk. Kem. Tid.*, **43**, 252 (1931); **44**, 169, 235 (1932).

²⁶ Mokrishin, *J. Gen. Chem. (U.S.S.R.)*, **1**, 856 (1931); **2**, 911 (1932).

²⁷ Prasad, *J. Indian Chem. Soc.*, **10**, 135 (1933).

²⁸ van Arkel, *Rec. trav. chim.*, **53**, 246 (1934).

of the causes which lead to variations in the properties of the sort mentioned above are either lacking or uncertain. It must not be construed from these statements that careful studies of such properties as surface tension are without value; on the contrary, their practical importance is well established.

If we write the well-known van der Waals' equation in the form

$$P = \frac{RT}{V - b} - \frac{a}{V^2}$$

where V is the molal volume of the liquid, it is seen that there are two independent factors which contribute to the total pressure, P . The first expression on the right is positive and is interpreted as the pressure due to the thermal agitation of the molecules; the second expression is negative, i.e., tends to counteract the effect of thermal agitation, and is due to the attractive forces exerted between the molecules. The term a/V^2 , where a is a constant and V is the molal volume, is defined as the internal pressure. It must be remembered that the internal pressure is exerted inward, into the body of the liquid, and is different in this respect from pressures as they are ordinarily considered. The internal pressure a/V^2 , may be approximated by an expression more conveniently measured, L/V , where L is the molal heat of vaporization and V is the molal volume. Table V gives the internal pressures of some organic liquids as tabulated by Mortimer.²⁹

TABLE V
RELATIVE INTERNAL PRESSURES

Compound	Relative Internal Pressures	Compound	Relative Internal Pressures
Hexane.....	0.56	Naphthalene.....	1.00
Diethyl ether.....	0.62	<i>p</i> -Dibromobenzene.....	1.09
Ethyl acetate.....	0.73	Ethylene dibromide....	1.13
Carbon tetrachloride...	0.81	Pyridine.....	1.17
Toluene.....	0.90	Carbon disulfide.....	1.18
Benzene.....	0.96	Phenol.....	1.4
		Water.....	4.5

By association is meant the coalescence of several molecules of the same species to a degree of firmness which enables the group to act as a unit during certain physical measurements. The degree of association of

²⁹ Mortimer, *J. Am. Chem. Soc.*, **44**, 1416 (1922).

various substances is determined by a variety of factors not clearly understood, but generally is greater for polar than for non-polar compounds. Molecular association is frequently responsible for apparent deviations from generalized rules applying to other properties; thus, it is often responsible for boiling points being higher than predicted and for similar increases in viscosity, density, and refractive index. Longinescu³⁰ has pointed out an empirical relationship between the molecular volumes and the degrees of association of some liquids. If the molal concentration of a liquid (C_m) be defined as the number of gram molecules per liter, the degree of association is nearly equal to $C_m/10$.

TABLE VI
COMPARISON OF MOLECULAR ASSOCIATION AND MOLAL CONCENTRATION

Compound	Degree of Association	$C_m/10$	Compound	Degree of Association	$C_m/10$
Ethyl alcohol	1.8	1.71	Nitroethane	1.40	1.39
Ethyl ether	0.99	0.99	Toluene	0.94	0.94
Propyl alcohol	1.4	1.39	Aniline	1.05	1.10
Ethyl acetate	1.08	1.05	Benzonitrile	0.97	0.97
Methyl formate	1.62	1.60	Chlorobenzene	0.99	0.98
Acetic anhydride	1.04	1.06	Bromobenzene	0.94	0.95
Carbon tetrachloride	1.01	1.04	Phenol	1.13	1.13
			Chloral	1.02	1.02

The degree of association for each compound in Table VI has been calculated from the Ramsay-Shields equation (*vide infra*) by considering deviations from the simple law. This procedure is not universally legitimate but appears to be satisfactory when applied to systems of simple molecules. The values of the degree of association in Table VI which are not integers obviously do not imply that a fraction of a molecule enters into combination with another; a non-integral value means that the actual number of particles (molecules plus complexes) is less than the number calculated from Avogadro's law by the ratio (calculated value)/(degree of association measured).

The solubility of one substance in another is determined largely by the relative intermolecular forces between the like and unlike molecules. Since internal pressures are a measure of these intermolecular forces, it is

³⁰ Longinescu, *Chem. Rev.*, **6**, 410 (1929).

to be expected that substances with internal pressures nearly equal will be more miscible than those whose internal pressures differ appreciably from each other. The differences in solubility of a solute in a series of solvents will therefore be determined by the differences between the internal pressures of the solute and the various solvents. Mortimer³¹ has tabulated data on the solubility of *p*-dibromobenzene in a variety of solvents of different internal pressures.

TABLE VII
SOLUBILITY OF *p*-DIBROMOBENZENE

Solvent	Internal Pressure	Solubility, Moles Solute per Liter of Solvent
Hexane	0.56	8.6×10^{-2}
Diethyl ether	0.62	18.3
Carbon tetrachloride	0.81	19.3
Benzene	0.96	21.7
<i>p</i> -Dibromobenzene	1.09	(24.8)
Carbon disulfide	1.18	22.4
Nitrobenzene	1.07	17.4
Aniline	1.4	10.7
Phenol	1.4	4.67
Ethyl alcohol	2.9	1.98

In Table VII it will be noted that the ideal solubility of 24.8, i.e., the solubility of *p*-dibromobenzene in pure *p*-dibromobenzene, is approached when the internal pressure of the solvent is nearly that of *p*-dibromobenzene. When the internal pressure of the solvent differs greatly from that of *p*-dibromobenzene, the solubility of the latter is relatively low.

Since the internal pressure of a polar compound is usually greater than that of a non-polar compound, a polar compound is less soluble in a non-polar than in a polar solvent. Similarly, non-polar solutes are less soluble in polar than in non-polar solvents. A change in the structure of a compound which likewise changes its polarity will alter appreciably the solubility behavior of the compound. Thus, in the alcohol and fatty acid series the lower, polar members are soluble in water while the higher, less polar members are soluble in the more non-polar solvents such as hydrocarbons.

Hildebrand^{32,33} has pointed out that Raoult's law can sometimes

³¹ Mortimer, *J. Am. Chem. Soc.*, **45**, 633 (1923).

³² Hildebrand, *ibid.*, **51**, 66 (1929).

³³ Hildebrand, *ibid.*, **37**, 970 (1915).

be utilized in the determination of solubilities. Liquid substances showing no heats of solution or no deviations from additivity of volumes on solution in general obey Raoult's law; the evolution of heat or a decrease of volume upon dissolving one substance in another usually indicates a negative deviation from the law, whereas changes in the opposite direction indicate a positive deviation. If two substances have essentially the same heat of solution, their solubilities in a given solvent will be in the order of their melting points.

Surface tension is the most easily determined of the properties which are strongly dependent upon intermolecular attraction. Surface tension

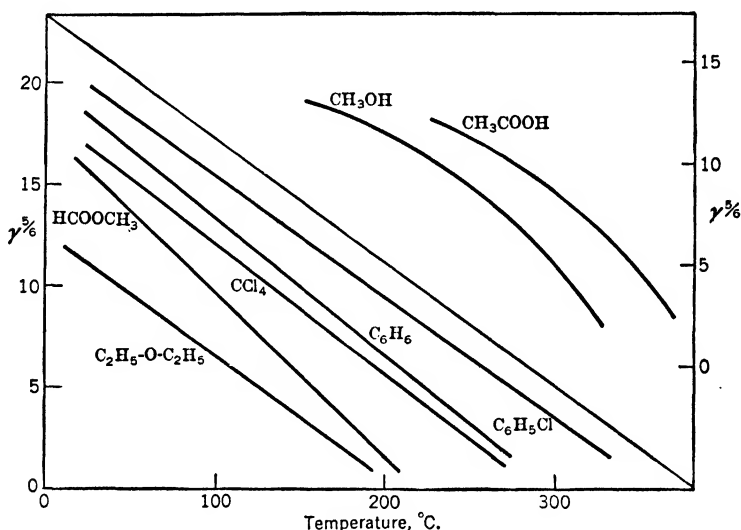


FIG. 5.*—Relation between surface tension and temperature.

is a measure of the amount of work done in forming the surface of a liquid, that is, in overcoming the internal attractive forces and bringing the molecules to the surface. Although this property is conveniently measured for liquids, no satisfactory method has been devised for determining the surface tension of solids.

Several empirical equations relating temperature and surface tension have been proposed; in one of these equations the five-sixths power of the surface tension is made proportional to the temperature. In Fig. 5 (taken from Sugden³⁴), where $\gamma^{5/6}$ is plotted against T for several substances, it is seen that many liquids obey the exponential law whereas

* Taken from Sugden, *loc. cit.* (Courtesy of publishers.)

³⁴ Sugden, "The Parachor and Valency," Routledge and Sons, Ltd., London (1930).

certain compounds such as methyl alcohol and acetic acid deviate from it. These compounds which do not follow the law are associated, and the deviations are probably due to changes in degrees of association with temperature.³⁵

The most general relation between surface tension and temperature is that of Eötvös, Ramsay, and Shields. Their equation holds for non-associated liquids up to the critical temperature and is expressed as follows:

$$\gamma \left(\frac{M}{D - d} \right)^{2/3} = K(T_c - T)$$

where γ is the surface tension, M the molecular weight, D and d the densities of the liquid and gaseous forms of the substance, T_c the critical temperature, T the temperature of observation, and K a constant. The constant K is usually about 2.1 for non-associated liquids when the temperature is expressed in degrees centigrade.

The analysis of surface tension data has not led to the satisfactory assignment of additive and constitutive values for various atomic groups and linkages. This is due, as mentioned before, to the lack of understanding of the effect of these factors on intermolecular forces. It is well known that molecular orientation at the surface plays a large part in the determination of the surface tension of a liquid. It has been found³⁶ that the molecules of organic liquids are oriented at the surface in much the same manner as atoms and molecules in crystals. This ordered arrangement has made it possible to examine organic liquids by x-ray reflection measurements and to determine, for some molecules, their configurations and dimensions.

The surface tension between two liquids, called the interfacial tension, is determined by the surface tension of each and by the attractive forces exerted between the unlike molecules. Bartell³⁷ reports that when two liquids are mutually insoluble, e.g., in systems comprising an organic liquid and mercury, the individual interfacial tension values between one liquid and the members of an homologous series decrease progressively if the surface tension values of the members of the series increase progressively. With a strongly polar substance like water as one of the interfacial liquids, the interfacial tension of aliphatic compounds decreases in the order: saturated hydrocarbons, unsaturated hydrocarbons, alkyl halides, esters, ethers, ketones, aldehydes, amines, alcohols, acids.

³⁵ Barbulescu, *J. chim. phys.*, **29**, 418 (1932).

³⁶ Stewart, *Rev. Modern Phys.*, **2**, 116 (1930).

³⁷ Bartell and co-workers, *J. Am. Chem. Soc.*, **55**, 2419 (1933).

TABLE VIII
INTERFACIAL TENSIONS (Dynes/cm.)

Phase 1	Phase 2		
	Air-vapor	Water	Hg
Water.....	72.75	375
Benzene.....	28.86	35.00	364.3
Toluene.....	36.1	363.6
Chloroform.....	27.1	32.80	357
Aniline.....	42.56	5.8	341
Diethyl ether.....	17	10.7	379
Nitrobenzene.....	43.38	25.66	349
<i>n</i> -Hexane.....	18.43	51.1	379.9
<i>n</i> -Heptane.....	378.7
<i>n</i> -Octane.....	21.77	50.81	376.0

Density, Molecular Volume, Parachor, and Nullpunktsvolume. The actual volume of the molecules of a gas at moderate pressures is relatively small compared to the total volume occupied by the gas. Intermolecular forces are therefore considerably weaker in the gaseous than in the liquid state, and those properties of liquids which are determined almost entirely by such forces are often unimportant in a gas. On the other hand, properties which do not depend primarily upon intermolecular forces are usually most accurately measured in the gaseous state. Conditions similar to those which hold in a gas are often approached in dilute solutions when the solvent is chemically inert and electrically non-polar.

The apparent size of a molecule in the gaseous state depends upon the method of measurement. Thus, if the molecular size of a substance is determined by means of viscosity measurements, a value is obtained which is a measure of the distance from the center of the molecule to a shell of frictional drag; if the diameter is measured by a method which depends upon the number of collisions undergone by the molecule, a figure is obtained which is an expression of the distance from its center to the point of nearest approach of the colliding particle.

Molecular sizes are additive in the sense that they can be calculated from the equilibrium distances of atoms in various types of linkages and from known bond angles.

The gram-molecular volumes of organic compounds in the liquid and solid state are defined as $M_v = M/d$, when M_r is the molecular volume,

M the molecular weight, and d the density. Two factors which markedly influence the molecular volume of a substance are temperature and internal pressure. In order to determine the additive and constitutive elements which constitute total molecular volume it is necessary to eliminate intermolecular attraction as much as possible. This has been accomplished by measuring the partial molal volumes of substances in dilute solutions of inert solvents. From measurements of partial molal volumes of various compounds in the same solvent and at the same dilution, Traube³⁸ concluded that in dilute solutions molar volumes, or molecular solution volumes as he termed them, could be calculated from atomic constants. Recent work³⁹ has applied this principle of molecular volumes observed in dilute solutions to the calculation of additive constants with a considerable degree of success.

An excellent example of the utility of the systematic study of a physical property for purposes of predicting and checking numerical values of the property is afforded by a study of the molal volumes of normal alkanes as reported by Calingaert and Hladky.¹⁴

A highly successful method of taking account of intermolecular forces in calculating additive and constitutive factors of molecular volumes has been developed by Sugden.⁴⁰ Macleod observed the empirical relationship $\gamma = C(D-d)^4$, where γ is the surface tension of a compound, D its density in the liquid form, d its density in the vapor form, and C a constant. Sugden multiplied this expression by M , the molecular weight, and obtained

$$\frac{M\gamma^{1/4}}{D-d} = MC = P$$

where P is called the parachor.

At the critical temperature, the surface tension of a compound is zero, since the intermolecular attractive forces are equal to the thermal kinetic forces. Since $\gamma = 0$ for all compounds at their critical temperatures, molecular volumes measured at these temperatures are more strictly comparable when additive and constitutive factors are being investigated. Sugden has pointed out that the parachor for most substances is equal to 0.77 times the molecular volume at the critical temperature, i.e., $P/V_c = 0.77$.*

³⁸ Traube, "Samml. chem. chem.-tech. Vorträge," Vol. 4, p. 255 (1899).

³⁹ Cohn, McMeekin, Edsall, and Blanchard, *J. Am. Chem. Soc.*, **56**, 784 (1934).

⁴⁰ Sugden, "The Parachor and Valency," Routledge and Sons, Ltd., London (1930).

* It would seem at first sight that P should equal zero at the critical temperature since $\gamma = 0$. This is not necessarily true, because $D-d$ also equals zero and the quotient of $\gamma^{1/4}$ and $D-d$ is indeterminate. There is therefore no reason to expect a sudden discontinuity in the value of P as the temperature approaches the critical temperature.

In Table IX are found data collected by Sugden. In this table, P is the empirically calculated parachor, V_c is the molecular volume at the critical temperature, and a is the molecular area as calculated from collision numbers.

TABLE IX
PARACHORS, CRITICAL VOLUMES, AND MEAN COLLISION AREAS

Compound	P	V_c	P/V_c	$a(\times 10^{16})$ cm ²	$P^{3/2}/a$
Propyl acetate.....	256.1	345.3	0.74	17.11	2.30×10^{16}
Ethyl acetate.....	217.1	286.3	0.76	15.01	2.41
Methyl acetate.....	177.0	227.8	0.78	12.80
Methane.....	73.2	99	0.74	7.72	2.27
Ethane.....	110.5	143	0.77
Ethylene.....	99.5	127	0.78	10.81	2.16
Methyl chloride.....	110.4	136	0.81	9.67	2.38
Ethyl chloride.....	149.4	194	0.77	11.94	2.36
Methyl formate.....	138.6	172.0	0.81	17.11	2.30
Ethyl propionate.....	254.8	344.3	0.74
Diethyl ether.....	211.7	281.9	0.75
Benzene.....	206.3	256.1	0.81	13.83	2.52
Chlorobenzene.....	244.5	307.8	0.80
		Mean	0.77	Mean	2.32

The analysis of parachor values for a great number of compounds has proved highly successful. Perhaps no empirical function except molecular refraction is at present as important for purposes of structure identification as the parachor. Much work has been done and is being done to arrive at satisfactory numerical parachor values for the various structural units which comprise most organic compounds. Sippel⁴¹ has prepared a set of atomic constants for non-cyclic compounds and a series of increment values for cyclic compounds; reasonably satisfactory values are obtained by his method, although no reference is made to the linking of the various atoms. Mumford and Phillips⁴² have revised Sugden's constants to correct errors in the parachors of higher homologs and have considerably improved the accuracy of the calculated values as compared to those observed. Vogel⁴³ has recently revised some of Sugden's original values.

⁴¹ Sippel, *Ber.*, **63**, 2185 (1930).

⁴² Mumford and Phillips, *J. Chem. Soc.*, 2112 (1929).

⁴³ Vogel, *ibid.*, 333 (1934).

Parachor values for some structural units as calculated by various investigators are collected in Table X. A computation of the parachor

TABLE X

ATOMIC AND STRUCTURAL CONSTANTS FOR CALCULATION OF THE PARACHOR

	S.*	M.P.	V.
CH ₂	39.0	40.0	40.3
C.....	4.8	9.2	11.5
H.....	17.1	15.4	14.4
O.....	20.0	20.0
O ₂ (Ester).....	60.0
N.....	12.5	17.5
S.....	48.2	50.0
F.....	25.7	25.5
Cl.....	54.3	55.0
Br.....	68.0	69.0
I.....	91.0	90.0
Single bond.....	-9.5
Double bond.....	23.2	19.0
Triple bond.....	46.6	38.0
3-Membered ring.....	16.7	12.5
4- " ".....	11.6	6.0
5- " ".....	8.5	3.0
6- " ".....	6.1	0.8
7- " ".....	-4.0

* S. = Sugden; M. P. = Mumford and Phillips; V. = Vogel.

value for benzene illustrates the use of the data in Table X. Benzene has 6 carbon atoms, 6 hydrogen atoms, and 3 double bonds; it is a six membered ring. Using Mumford and Phillips' values as given in the table, we find:

$$P = (6 \times 9.2) + (6 \times 15.4) + (3 \times 19) + 0.8 = 205.4$$

The value computed from the experimental observations is 206.0.

If the molecules of a liquid or solid are subjected to extremely high pressures or very low temperatures, the effect of thermal repulsive forces on the molecular volume is reduced to a considerable extent. Biltz⁴⁴ and his co-workers have discussed the relationships between the nullpunkts-volume, calculated empirically by extrapolation of the temperature-density curves to absolute zero, and the constitution of organic com-

⁴⁴ Biltz and co-workers, *Z. physik. Chem.*, **A151**, 13 (1930), and other papers in this series.

pounds in the liquid and solid states. The analysis of the data has led to constitutive and additive numerical constants which gave reasonably satisfactory values when compared to those observed. Table XI lists a number of compounds for which the nullpunktsvolumes have been determined; experimentally determined and empirically calculated values for this quantity are given. Included in the table are the corresponding parachor values.

TABLE XI
PARACHOR AND NULLPUNKTSVOLUME VALUES OF SOME TYPICAL
ORGANIC COMPOUNDS

Compound	Nullpunktsvolume			P (using M. and P.'s Factors; cf. Table X)		
	Calc.	Obs.	Δ	Calc.	Obs.	Δ
Methyl alcohol.....	30.2	30.5	+0.3	85.4	88.3	+2.9
Ethyl alcohol.....	45.2	44.7	-0.5	125.4	126.8	+1.4
Ethane.....	41.6	40.0	-1.6	110.8	110.5	-0.3
Formic acid.....	29.5	29.1	-0.4
Acetic acid.....	44.5	44.7	+0.2	130.6	131.2	+0.6
Acetone.....	55.9	55.2	-0.7	159.0	161.5	+2.5
Acetaldehyde.....	40.9	38.3	-2.6
Ethyl ether.....	75.2	75.8	+0.6	211.3	211.7	+0.4
Stearic acid.....	770.6	778.0	+7.4
Cyclohexanol.....	255.4	254.9	-0.5
Benzene.....	67.2	69.3	+2.1	205.4	206.0	+0.6
Naphthalene.....	100.4	102.5	+2.1
Anthracene.....	133.6	135.8	+2.2
Toluene.....	82.2	83.6	+1.4	245.4	246.2	+0.8
Xylene.....	97.2	98.2	+1.0	285.4	283.8	-1.6
Mesitylene.....	112.2	111.2	-1.0	321.8	320.8	-1.0
Ethylbenzene.....	97.2	97.7	+0.5
Propylbenzene.....	112.2	116.2	+4.0
Benzaldehyde.....	81.5	82.4	+0.9	253.6	255.1	+1.5
Methyl salicylate....	321.8	323.7	+1.9

In Table XI it will be noticed that calculated nullpunktsvolume values for aliphatic compounds are for the most part greater than the observed values; for aromatic compounds the calculated values are uniformly lower than the observed. Additional constitutive factors which take account of ring formation, steric effects, etc., must be introduced in the calculations if better agreement between observed and computed values is to be realized. Ruzicka⁴⁵ states that ring compounds

⁴⁵ Ruzicka and co-workers, *Helv. Chim. Acta*, **16**, 487 (1933).

with eight or more carbon atoms have parachor and compressibility values which indicate that the compounds are aliphatic in character.

The influence of the crystal form of solid substances on their nullpunktsvolumes is illustrated in Fig. 6, which graphically presents the nullpunktsvolumes of members of two homologous series. It is seen that the alternating effect of the even and odd numbers of the series which is characteristic of melting points is also found in the volume values of solid compounds. The molecular volumes and parachors of corresponding liquid substances do not show this alternating effect.

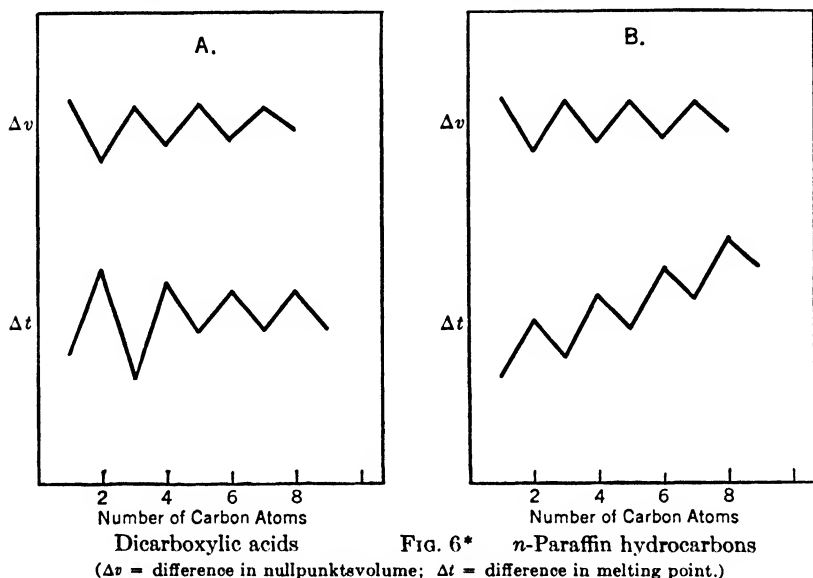


FIG. 6* n-Paraffin hydrocarbons

(Δv = difference in nullpunktsvolume; Δt = difference in melting point.)

Viscosity. The viscosity of a liquid is a measure of the resistance set up by intermolecular attractive forces to the passage of one molecule past another. Viscosity data are therefore subject to the same difficulties of analysis as those of other properties which depend upon the mutual attraction of like molecules. Viscosities are strongly dependent upon temperature, and measurements designed to separate the additive and constitutive components of this property must be carried out under conditions which are equivalent for all compounds.

Some measure of success has been obtained in efforts to prepare series of numerical constants from which molecular viscosities can be calculated. Dunstan and Thole⁴⁶ have developed the formula $\log \eta = aM + b$, where η is the viscosity, M the molecular weight, a a general constant,

* Taken from Biltz, Fischer, and Wünnenberg, *Z. physik. Chem.*, **A151**, (1930). (Courtesy of publishers.)

⁴⁶ Dunstan and Thole, *J. Inst. Petroleum Tech.*, **4**, 191 (1918).

and b a constant characteristic of an homologous series. By the use of this formula, logarithmic increments for various atomic groups have been calculated.

TABLE XII

INCREMENT FACTORS FOR VISCOSITY PREDICTION (Dunstan and Thole)

CH ₂	0.107	Alcoholic O.....	2.12
Aliphatic H.....	0.934	Double bond.....	1.847
Aliphatic O.....	0.098	<i>n</i> -Carbon.....	-1.761
<i>Iso</i> -Carbon.....	-0.030		

Fluidity, which is the reciprocal of the viscosity, has been shown⁴⁷ to be a function of the vapor pressure. At high vapor pressures the relationship is nearly linear, and from data taken in the range where this linearity holds it has been possible to calculate increment factors for fluidity.

TABLE XIII

INCREMENT FACTORS FOR PREDICTION OF FLUIDITY (Bingham)

	Fluidity		Fluidity
Carbon.....	-95	Hydrogen.....	59
Oxygen.....	24	<i>Iso</i> -linkage.....	-76
Double bond.....	114	Sulfur.....	76
Chlorine.....	109		

Prasad⁴⁸ has observed regularities in the temperature-viscosity relationships of members of homologous series. He finds indications that the odd and even members of the series fall into separate groups.

Staudinger, in his studies on the viscosities of solutions of high-molecular-weight compounds,⁴⁹ has introduced the term specific viscosity, $\eta_{sp.}$, which is defined as the increase in viscosity which is produced in a solvent by dissolving a unit amount of a substance in a unit volume of the solvent. He has made use of the expression $\eta_{sp.}/C = KL$, where C is the concentration of a primary molal solution (1.4 per cent = CH₂/1000), K a constant, and L the length of the carbon chain in Ångström units of the material being investigated. For simple, normal organic compounds such as paraffins, fatty acids, etc., the specific viscosity of a primary molal solution may be expressed as $\eta_{sp.}$ (1.4 per cent) = $ny + x$, where n is the number of carbon atoms in the chain, y the viscosity of a single carbon atom, and x that of oxygen. For many compounds y is approximately constant. The plot of $\eta_{sp.}/C$ against

⁴⁷ Bingham and Spooner, *J. Rheol.*, **3**, 221 (1932).

⁴⁸ Prasad, *J. Indian Chem. Soc.*, **10**, 143 (1933).

⁴⁹ Staudinger, "Die hochmolekularen organischen Verbindungen," Springer, Berlin (1932).

the number of carbon atoms for several homologous series shows that the slopes of the lines are approximately the same; the intercepts on the η_{sp}/C axis therefore give the values of x for each series. Some of Staudinger's results are shown in Fig. 7.

Staudinger has extended his concept of additive group viscosity values to considerations of the viscosities of high-molecular-weight polymers such as cellulose and its derivatives (p. 1585). From his data he has calculated the molecular weights of such compounds and has

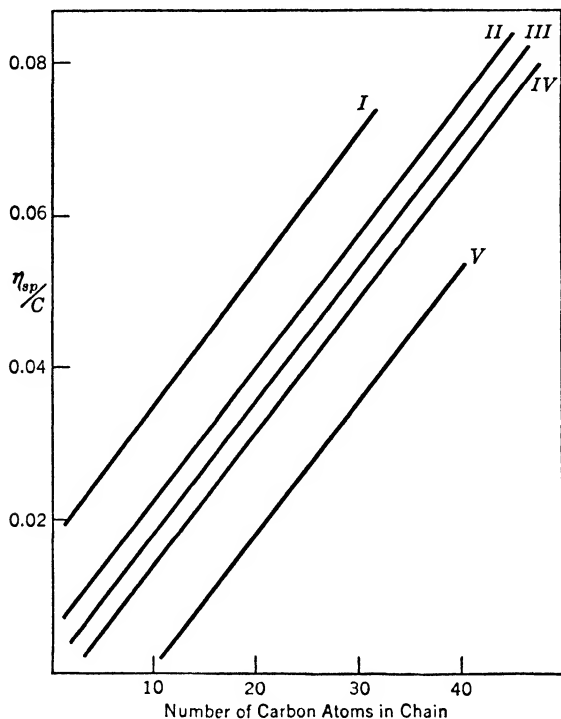


FIG. 7.*—Viscosity changes in homologous series.

I = *n*-fatty acids in pyridine; *II* = *n*-fatty acids; *III* = *n*-fatty acid esters (ethyl);
IV = *n*-paraffins; *V* = *n*-alcohols.

computed the numbers of structural units in the polymeric chains. Some of his results do not agree with values obtained by other methods of measurement.

Refractive Index and Molecular Refraction. The effect of molecular structure upon refractive index has been extensively studied,⁵⁰ and the

* From Staudinger, *loc. cit.*, p. 63. (Courtesy of publisher.)

⁵⁰ (a) Cohen, "Organic Chemistry for Advanced Students," Edward Arnold and Co., London (1928), Vol. II, pp. 19–45. (b) Henrich, Johnson, and Hahn, "Theories of Organic Chemistry," John Wiley and Sons, New York (1922), pp. 288–317.

data have been analyzed more successfully than those for most physical properties. The accuracy with which the refractive index of a substance can be measured and the ability to make observations at various wave lengths have provided sufficiently reliable data to permit the calculation of reasonably consistent additive and constitutive factors for the various positions and types of atomic linkages.

The refractive index, n , of a substance is the ratio of the velocity of light in vacuum to the velocity of light in that substance. It is a measure of the interaction of the electrostatic and electromagnetic fields set up by the atoms in the material with the electromagnetic and electrostatic components of the traversing light waves and is dependent upon the intermolecular attractive forces which determine molecular volume and internal pressure. The refractive index is independent of temperature except for thermal expansion effects.

A useful function called the specific refractivity, r , has been developed to take account of the dependence of the refractive index upon specific volume. The specific refractivity or specific refraction, r , has been defined as $r = \frac{n - 1}{d}$, where d is the density. Lorenz and Lorentz⁵¹

have proposed the formula, $r = \frac{n^2 - 1}{n^2 + 2} \cdot \frac{1}{d}$, as being more nearly independent of pressure and temperature, and their expression is the more commonly used of the two. The molecular refraction, M , is determined by multiplying the specific refraction by the molecular weight.

The refractive index varies with the wave length of light, and it is therefore necessary to designate the wave length at which the measurement is made. This is customarily done by using a subscript; thus η_α indicates the refractive index as observed with the hydrogen α line, M_D the molecular refraction as measured with the sodium D line and $M_{546.1}$ the molecular refraction at λ 546.1 m μ (the mercury green line). The difference between molecular refractions measured at two wave lengths is called the molecular dispersion, and is designated as $M_\alpha - M_\beta$; the difference between the specific refractions measured at several wave lengths has been designated by Auwers⁵² as $\Sigma_{\alpha-\beta}$ or $\Sigma_\alpha - \Sigma_\beta$. Auwers and Eisenlohr⁵³ usually express the specific refractive dispersion (Σ) as 100 times the observed value in order to yield a factor of convenient size. Hückel⁵⁴ has suggested that the molecular dispersion rather

⁵¹ (a) Lorentz, *Wied. Ann.*, **9**, 641 (1880). (b) Lorenz, *ibid.*, **11**, 70 (1880).

⁵² Auwers and Eisenlohr, *Ber.*, **43**, 806 (1910), and subsequent articles in this journal, *Ann.*, and *J. prakt. Chem.*

⁵³ Eisenlohr, "Spektrochemie organischer Verbindungen," Enke, Stuttgart (1912).

⁵⁴ Hückel, *Z. physik. Chem.*, **A163**, 67 (1932).

than the specific dispersion should be used in comparison of refractive properties.⁵⁵

The mathematical analysis of accurate data has led to the assignment of refraction values for the atomic components which constitute most organic compounds. The addition of these values frequently leads to molecular refractions in agreement with the observed. In Table XIV are given some of the atomic factors suggested by Auwers and Eisenlohr.

TABLE XIV
ATOMIC FACTORS FOR CALCULATING MOLECULAR REFRACTIONS

Group	H _α	D	H _β	H _γ	H _γ —H _α
—CH ₂ —	4.598	4.618	4.668	4.710	0.113
C	2.413	2.418	2.438	2.466	0.056
H	1.092	1.100	1.115	1.122	0.029
O(carbonyl)	2.189	2.211	2.247	2.267	0.078
O(ether)	1.639	1.643	1.649	1.662	0.019
O(hydroxyl)	1.522	1.525	1.531	1.541	0.015
Cl	5.933	5.967	6.043	6.101	0.168
Br	8.803	8.865	8.999	9.152	0.340
I	13.757	13.900	14.224	14.521	0.775
N(<i>pri.</i> -amine)	2.309	2.322	2.368	2.397	0.086
N(<i>sec.</i> -amine)	2.475	2.499	2.561	2.603	0.119
N(<i>tert.</i> -amine)	2.807	2.840	2.940	3.000	0.186
S(mercaptan)	7.63	7.69	7.83	7.98	0.35
CN	5.434	5.459
C=C(double bond)	1.686	1.733	1.824	1.893	0.200
C≡C(triple bond)	2.328	2.398	2.506	2.538	0.171

The headings in columns 2, 3, 4, 5, and 6 of Table XIV indicate the wave lengths of light used in the measurements.

Ruzicka *et al.*^{56, 57, 58} have suggested that ring increment values, based on a six-membered ring as unity, should be applied in calculating refractions as follows: five-membered ring, +0.04; six-membered ring, 0.00; seven-membered ring, -0.10; eight-membered ring, -0.47; 15-membered ring, -0.62; etc.

When the refractions of some compounds are computed from the various atomic factors, it is found that there are differences between these values and those observed experimentally. The exaltations or

⁵⁵ Auwers, *ibid.*, **A164**, 44 (1933).

⁵⁶ Straus and Kühnel, *Ber.*, **65**, 154 (1932); Auwers, *Ber.*, **65**, 146 (1932).

⁵⁷ Ruzicka, *Helv. Chim. Acta*, **14**, 1319 (1931).

⁵⁸ Bruylants and Merckx, *Bull. soc. chim. Belg.*, **43**, 248 (1934).

depressions of the observed from the calculated refraction values have been studied for a number of compounds, and postulates have been made as to the causes of the variations. From the results of such study it has been possible to assign definite structures to certain molecules. The exaltation of the molecular refraction is usually indicated as EM (for the α hydrogen line or, for a given line $\beta, \gamma \dots$ etc.: $EM_\beta, EM_\gamma \dots$ etc.). The exaltation of the molecular or specific dispersion is indicated in a similar manner, i.e., $E(M'_\gamma - M_\alpha)$ or $EM_{\gamma-\alpha}$ and $E(\Sigma_\gamma - \Sigma_\alpha)$ or $E\Sigma_{\gamma-\alpha}$. Auwers has found it preferable to express these latter values in a percentage form, since the results are then independent of the particular function of refractive index used.

From the analysis of exaltation data, certain generalizations have been reached concerning the effect of unsaturation (p. 543) on molecular refraction. One double bond in a compound produces no exaltation beyond that involved in the double bond increment used in the calculation of the theoretical molecular refraction. The presence of more than one double bond in a molecule similarly produces no exaltation provided the double bonds are not conjugated. The presence of conjugation in the molecule produces distinct exaltation, the amount being influenced by the length of the conjugated system, the presence of substituents, and the branching of the conjugated chain. Examples of these effects are presented in Table XV.

From this table it is seen that the exaltation increases as the length of the conjugated chain becomes greater provided the chain is unsubstituted. For a given chain the exaltation decreases progressively as the carbon atoms are progressively substituted with R groups. Exaltation is greatest for a linear conjugated system and decreases as the system becomes more and more branched.

In a study of the exaltation of methylbutadiene derivatives⁵⁹ it was observed that the shifting of the methyl substituents to the center of the conjugated system resulted in a marked lowering of the exaltation.

There are certain exceptions to the general exaltation of conjugated linkages; for example, the exaltations of benzene, furan, thiophene, pyrrole, and cyclopentadiene are nearly zero. The identity of the calculated and observed molecular dispersion values for these compounds is explained as being due to the existence of two opposing factors which nearly balance, that of conjugation producing a positive exaltation and that of ring strain (p. 4) or ring closure producing a negative exaltation. Although benzene, cyclopentadiene, and similar monocyclic compounds show practically no exaltation, the polycyclic compounds derived

⁵⁹ Farmer and Warren, *J. Chem. Soc.*, 3221 (1931).

TABLE XV



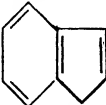


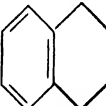

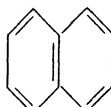
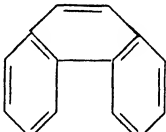
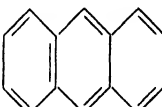
INFLUENCE OF CONJUGATION ON REFRACTION

(a) Length of Conjugation	$E\Sigma_{\gamma-\alpha}$	$E\Sigma$ (Per Cent)
$-\text{CH}=\text{CH}-$	0.33	0
$-\text{CH}=\text{CH}-\text{CH}=\text{CH}-$	1.90	50
$-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{CH}=\text{CH}-$	4.20	160
(b) Substitutions on Conjugated Chain		
$-\text{CH}=\text{CH}-\text{CH}=\text{CH}-$	1.90	50
$\begin{array}{c} \text{R} \\ \\ -\text{CH}=\text{CH}-\text{C}=\text{CH}- \end{array}$	1.10	45
$\begin{array}{c} \text{R} \quad \text{R} \\ \quad \\ -\text{CH}=\text{C}-\text{C}=\text{CH}- \end{array}$	0.90	35
$\begin{array}{c} =\text{CH} \\ \text{(arom.)} \diagup \\ -\text{CH}=\text{C}-\text{CH}=\text{CH}- \end{array}$	1.10	45
$\begin{array}{c} =\text{CH} \\ \text{(arom.)} \diagup \\ -\text{CH}=\text{C}-\text{C}=\text{CH}- \\ \\ \text{R} \end{array}$	0.70	30
$\begin{array}{c} =\text{CH} \\ \text{(arom.)} \diagup \\ -\text{CH}=\text{C}-\text{C}=\text{C}- \\ \quad \\ \text{R} \quad \text{R} \end{array}$	0.45	20
$-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{OR} \end{array}$	2.4	120
$\begin{array}{c} \text{R} \\ \\ -\text{CH}=\text{C}-\text{CH}=\text{CH}-\text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{OR} \end{array} \end{array}$	2.0	100
$\begin{array}{c} \text{R} \quad \text{R} \\ \quad \\ -\text{CH}=\text{C}-\text{CH}=\text{C}-\text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{OR} \end{array} \end{array}$	1.5	75
(c) Branching of Conjugated Chains		
$-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{CH}=\text{CH}-$	4.20	160
$\begin{array}{c} \text{R} \quad \text{O} \quad \text{R} \\ \quad \quad \\ -\text{CH}=\text{C}-\text{C}-\text{C}=\text{CH}- \end{array}$	1.0	40
$-\text{CH}=\text{CH}-\text{CH}=\text{CH}-\text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{OR} \end{array}$	2.4	120
$\begin{array}{c} \text{O} \\ \\ -\text{CH}=\text{CH}-\text{CH}-\text{C} \begin{array}{l} \diagup \text{O} \\ \diagdown \text{OR} \end{array} \\ \\ =\text{CH} \end{array}$	0.5	25

by their combination show a decided $E\Sigma$; this fact has been interpreted as an indication that only one ring in such compounds is truly aromatic (p. 52), the other rings producing the exaltation characteristic of normal unsaturated conjugation.

TABLE XVI

RELATION BETWEEN CYCLIC STRUCTURE AND REFRACTION

		+		=	
	Benzene +6 per cent		Cyclopentadiene -0.5 per cent		Indene +35 per cent
$E\Sigma_{\gamma-\alpha} =$		+		=	
	Benzene +6 per cent		Cyclohexene 0.0 per cent		Tetrahydronaphthalene +16 per cent
$E\Sigma_{\gamma-\alpha} =$					
	Benzene +6 per cent	Naphthalene +60 per cent	Phenanthrene +90 per cent	Anthracene +170 per cent	

THERMODYNAMIC PROPERTIES OF ORGANIC COMPOUNDS

It has been mentioned earlier that the great value of the proper use of thermodynamic data lies in their prediction of the course and extent of chemical reactions. Recently, systematic investigations have been made of the thermodynamic properties of organic compounds with the result that certain regularities have been observed. Although the available data are still relatively meager for use in calculating equilibrium constants for a large number of organic reactions, the infallibility of the thermodynamic method warrants appreciation of its potentialities (p. 852).

The thermodynamic method is empirical in the sense that it is based upon certain arbitrary definitions and upon the experimentally proved infallibility of the so-called first, second, and third laws. By the mathematical analysis of the defined functions, this analysis being restricted by the necessity of observing the three thermodynamic laws, certain

relationships between experimentally determinable quantities are derived. It must be emphasized that the various thermodynamic properties such as free energy, heat content, and entropy are no more than mathematical functions which can be calculated from measurable quantities such as heats of reaction, equilibrium constants, and specific heats. The attempts of many chemists to define heat content, free energy, and entropy in a descriptive manner have often led to the mistaken impression that these quantities are associated with restricted physical processes. The thermodynamic functions can sometimes be very loosely interpreted in terms of physical processes, but their definitions are actually independent of such processes.

The chemist is usually interested in three thermodynamic functions: the heat content, H , or its change during reaction, ΔH ; the free energy, F , or the change in free energy, ΔF ; the entropy, S , or its change, ΔS . The change in heat content, ΔH , for a reaction is equal to the heat of the reaction measured at constant pressure; the heat content, H , for a substance at temperature T is equal to the heat capacity, C_p , of the substance measured at constant pressure and integrated from absolute zero to T . The entropy, S , of a compound is equal to the quotient C_p/T , integrated from absolute zero to T ; ΔS is the change in S attending a reaction. The change in free energy, ΔF , is defined by the equation $\Delta F = \Delta H - T\Delta S$; F is similarly defined by $F = H - TS$. The free energy change, ΔF , for a chemical reaction is a measure of the driving force of the reaction in the sense that its sign determines the direction of the reaction and its magnitude determines the equilibrium state; the usual equation expressing the relationship between ΔF and the extent of a reaction is $-\Delta F = RT \ln K$, where R is the gas constant, T the absolute temperature, and K the equilibrium constant. For the chemist, the values of F and ΔF are the most important of the three functions.

Parks and Huffman⁶⁰ have discussed in detail the thermodynamic properties of organic compounds. From measurements of the heat capacities of certain organic compounds and of their elements over a wide range of temperatures, entropies and heat contents have been calculated. These values, in conjunction with measured heats of reaction, have allowed the calculation of the free energies of formation of the compounds from their elements.

Parks and Huffman found that the entropies of the members of certain homologous series, e.g., the n -hydrocarbons, increase progres-

⁶⁰ (a) Parks and Huffman "Free Energies of Some Organic Compounds," Am. Chem. Soc. Monograph Series, No. 60, Chemical Catalog Co., New York (1932). (b) Rossini, *J. Research Nat. Bur. Standards*, **13**, 21, 189 (1934).

sively as the series is ascended (Fig. 8). The free energies of formation, ΔF , do not form a regular series, although variations are in the same direction and of the same order of magnitude. When alkyl groups are substituted for hydrogen in a n -hydrocarbon, the entropy of the branched-chain compound is less than that of the normal compound having the same number of carbon atoms. The formula $S_{298} = 25.0 + 7.7n - 4.5r$, where S_{298} is the entropy at 25° C., n is the number of carbon atoms in the compound, and r is the number of branched chains (alkyl) in the molecule, fits the data for a number of isomeric pentanes,

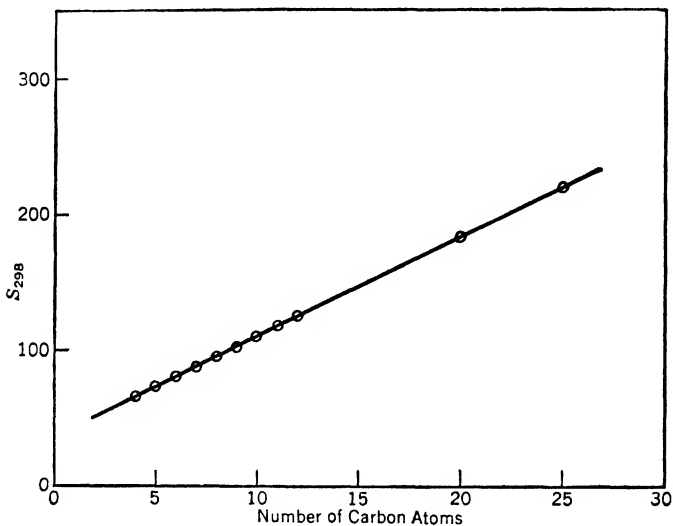


FIG. 8.*—Molal entropies of n -paraffins.

heptanes, and octanes satisfactorily. The heats of formation and free energies of formation at 25° C. vary in like manner with the introduction of side chains into the n -hydrocarbons; ΔH_{298} is lower for the branched chain compounds while ΔF_{298} is higher. The heats of formation and free energies of formation of unsaturated hydrocarbons are greater than those of the corresponding saturated compounds; the differences decrease as the unsaturation becomes buried in the molecule.

Three general equations have been given for the entropies of n -hydrocarbons in the liquid, gaseous, and solid states at 25° C.

$$S_{298} (\text{liquid}) = 25.0 + 7.7n$$

$$S_{298} (\text{gas}) = 34.0 + 10.0n$$

$$S_{298} (\text{solid}) = 18.0 + 5.8n$$

* From Parks and Huffman, *loc. cit.* (Courtesy of publishers.)

In these equations, n is the number of carbon atoms in the molecule. Parks and Huffman⁶¹ have considered organic compounds as derivatives of the hydrocarbons, and have prepared a list of substitution factors which, when added to the entropy values for the parent hydrocarbons, give corresponding entropies for the compounds. Their substitution factors are given in Table XVII.

TABLE XVII
MOLAL ENTROPY AND FREE ENERGY FACTORS

Structural Modifications	Change in Molal Entropy			Change in ΔF_{298}
	Solid	Liquid	Gas	
Addition of CH_2 in chain	5.8	7.7	10	1,080
Substitution of CH_3 for H (chain)	5.0	3.2	5.0	1,900
C_2H_5 for H (chain)	10.9	...	3,000
CH_3 for H (ring)	5.8	7.7
C_2H_5 for H (ring)	11.6	15.4	...	1,100
C_6H_5 for H	17.0	19.5	...	36,000
Cyclohexyl for H	26.5	...	13,000
Conversion $\text{C}-\text{C}$ to $\text{C}=\text{C}$	-2.7	-2.7	-2.7	20,000
Sub. OH for H (primary alcohol)	0	-1.5	13.0	-34,000
(secondary alcohol)	0.5	-4.0	9.0	-37,000
(tertiary alcohol)	0.5	-6.0	7.0	-41,000
(phenol)	0	0	...	-41,000
Sub. of $-\text{O}-$ to form ether	5.0	8.0	-20,000
Sub. of $=\text{O}$ for 2H (aldehyde)	1.0	5.0	...	-23,000
$=\text{O}$ for 2H (ketone)	1.0	0.5	6.0	-30,000
CO_2H for H (acid)	5.8	7.7	...	-83,200
NH_2 (amine)	0.0	0.0	...	6,000
NO_2 (nitro compd.)	7.0	8.0	...	7,000
Cl	6.0	7.0	9.0	-1,600
Br	7.5	9.0	11.5	4,500
I	9.0	11.0	14.0	10,000

In this table substitution factors have been given for the solid, liquid, and gaseous states. In comparing thermodynamic data to determine the effect of additive and constitutive factors, more consistent results are to be expected when the data apply to the gaseous state; this is due to the fact that intermolecular forces are almost completely absent in the gas.

⁶¹ Parks and Huffman, "Free Energies of Some Organic Compounds," Am. Chem. Soc. Monograph Series, No. 60, Chemical Catalog Co., New York (1932), p. 210.

The influence of steric effects on the heats of combustion of saturated and unsaturated hydrocarbons has been studied by Alder and Stein.⁶² The values given by Grimm and Wolf⁶³ as shown in Table XVIII for atomic and group factors to be used in the calculation of heats of formation for non-polar bonds have been used by Alder and Stein in their computations.

TABLE XVIII

SEPARATION ENERGY (HEAT OF FORMATION) FOR NON-POLAR BINDINGS
(IN KILOGRAM-CALORIES)

Binding	Aliphatic	Aromatic	Binding	Aliphatic	Aromatic
C—H	92	101	C—N	70	85
C—Cl	73	96	C≡N	212	..
C—Br	59	...	C—C	71	96
C—I	44	45	C=C	125	..
C—O—	92	109	C≡C	166	..
C=O	188	C(arom.)—C(aliph.)		80	

Kharasch⁶⁴ has compiled and evaluated the available combustion data on organic compounds and has developed a method of calculating the heats of combustion of liquid substances. His formula is $Q = 26.05n + C$, where Q is the heat of combustion, 26.05 the net amount of energy change per mol electron, n the number of valence electrons, and C a constant characteristic of the electron linkage. The values of the constant C for some linkages are given in Table XIX.

TABLE XIX

VALUES OF THE CONSTANT C FOR CALCULATING HEATS OF COMBUSTION

Coupling	C	Coupling	C
Aromatic to aliphatic.....	-3.5	Aromatic to aromatic.....	-6.5
Double bond		Triple Bond, one H.....	+46.1
Aliphatic, <i>cis</i>	16.5	no H.....	33.1
Aliphatic, <i>trans</i>	13.0	Primary alcohol.....	13.0
Aromatic to aliphatic.....	-6.5	Secondary alcohol.....	6.5
In ring.....	+6.5	Tertiary alcohol.....	3.5
Aldehyde (RCHO).....	13.0	Phenol.....	3.5
Ketone (RCOR).....	6.5	Ester (RCO ₂ R).....	16.5

⁶² Alder and Stein, *Ber.*, **67**, 613 (1934).

⁶³ Grimm and Wolf, *Handbuch der Physik*, **24**, 536 (1927).

⁶⁴ Kharasch, *Bur. Standards J. Research*, **2**, 359 (1929).

Kistiakowsky, Vaughan, *et al.*⁶⁵ have commenced a series of very accurate studies of the heats of hydrogenation of unsaturated hydrocarbons. They have found that with an increasing number of substituent alkyl groups the heat of hydrogenation of the ethylenic linkage is lowered in a progressive manner. Monosubstitution produces a decrease of 2.7 Cal. per mol; disubstitution (*cis*) 4.2 to (*trans*) 5.2; trisubstitution 5.9; and tetrasubstitution 6.2. The effect of substitution is independent of the chain length of the normal alkyl radical substituted, but branched chains appear to have some influence. The authors have made the very interesting observation that the first step in the hydrogenation of benzene is endothermic while the second and third steps are exothermic. This explains why it is not possible to hydrogenate benzene to the dihydro- or tetrahydroderivatives; the reaction proceeds past the first stage to the hexahydro compound.

Kistiakowsky and co-workers have found that the effect of conjugation in unsaturated hydrocarbons is to stabilize the double bond, the hydrogenations of 1,3-butadiene and 1,3-cyclohexadiene being less exothermic than the hydrogenation of ethylene.

Recent developments in experimental and theoretical studies of Raman spectra and band spectra have enabled calculations of free energies, entropies, and heat contents to be made from spectroscopic data. From such data, specific heats of elements and compounds can be computed, and from these, entropies and the other thermodynamic quantities. The results have been very satisfactory for simple substances, but difficulty is encountered when attempts are made to calculate the thermodynamic functions for polyatomic molecules. With the continually increasing study of these problems it is becoming possible to extend the limit of such calculations to molecules of greater and greater complexity.

PROPERTIES WHOSE ANALYSES LEAD DIRECTLY TO STRUCTURE DETERMINATION

Dipole Moment

The positions of the electrons in a molecule are determined by the relative electron affinities of the atoms which constitute it. If the molecule has an axis or plane of structural symmetry, the electrons will similarly be symmetrical about the axis or plane. Thus, in ethane or ethylene it is possible to divide the molecule into two halves, each of which is identical structurally and electronically (pp. 1608, 1864) with the other; similarly, cyclopropane has an axis of symmetry about which the three carbon atoms occupy identical relative positions. The

⁶⁵ Kistiakowsky, Vaughan, *et al.*, *J. Am. Chem. Soc.*, **55**, 146 (1936).

distribution of electrical charges in molecules such as ethane is symmetrical, with the result that the molecules as a whole are electrically balanced.

Most molecules have no plane or axis of structural symmetry, and it is generally true that the electrons in such molecules are likewise unsymmetrically placed. Owing to this lack of symmetry, there is usually a position in the molecule at which the density of electrons is greater than at other positions; this point of high electron concentration will be negatively charged with respect to the rest of the molecule which is relatively positive. If the total negative charge, $-e$, be considered localized at its center of gravity, a , and the corresponding positive charge, $+e$, be considered localized at point b , the electric moment of the molecule, μ , is defined as $e \times d$, where d is the distance from a to b .

The magnitude of the dipole moment, μ , is a measure of the electrical asymmetry of the molecule, and the study of such moments of organic compounds therefore affords a method of learning certain facts about molecular structure. In general, compounds with zero moment are symmetrical while substances which possess definite moments are polar, the greater the moment the larger the degree of polarity.

The study of the dipole moments of organic compounds has been extensively undertaken in recent years.⁶⁶ A variety of methods has been used for determining numerical values,^{67, 68} but that most widely used depends upon measurements of dielectric constants. The equation generally used for liquid substances is

$$\frac{d-1}{d+2} \cdot \frac{M}{\rho} = \left[1 - \frac{\lambda_o^2}{\lambda^2} \right] \left[\frac{n^2-1}{n^2+2} \right] \frac{M}{\rho} + \frac{4\pi N \mu^2}{9KT}$$

where d is the dielectric constant, M the molecular weight, ρ the density, n the refractive index, λ the wave length of measurement of n , λ_o the wave length of the absorption band nearest λ in the ultra-violet, N and K are Avogadro's and Boltzmann's constants, T the absolute temperature, and μ the electric moment. The values of μ for many substances are determined by making measurements in dilute solutions of electrically non-polar and inert solvents; this procedure is used in order to eliminate intermolecular effects. If experimentally feasible, dielectric constants are determined in the gaseous state because they are more reliable for interpretive purposes.

⁶⁶ (a) Smyth, "Dielectric Constants and Molecular Structure," Am. Chem. Soc. Monograph Series, No. 55, Chemical Catalog Co., New York (1931). (b) Debye, "Polar Molecules," Chemical Catalog Co., New York (1929). (c) Estermann, *Ergeb. exakt. Naturw.*, **8**, 258 (1929). (d) Sack, *ibid.*, **8**, 307 (1929). (e) Glasstone, "Recent Advances in Physical Chemistry," 2nd. ed., Blakiston's Son and Co., Philadelphia (1933).

⁶⁷ Estermann and Wohlwill, *Z. physik. Chem.*, **B20**, 195 (1933).

⁶⁸ van Arkel, *Rec. trav. chim.*, **52**, 719, 733 (1933).

The relationship between boiling points and dipole moments has been used to calculate the latter. Van Arkel⁶⁹ has shown that for symmetrical molecules of nearly spherical shape the Debye formula for calculating moments from boiling points is applicable provided there is no association. The boiling points of non-polar compounds can be computed from the atomic volumes of the constituent atoms. For example, for non-polar carbon-halogen molecules the equation used is

$$T_b = \frac{(V - V_c)}{V} \cdot K_a$$

In the equation T_b is the boiling point in absolute temperature, V and V_c the atomic volumes of the halogen and carbon, respectively, and K_a a constant involving the a of van der Waals' equation. The square root of a exhibits additive characteristics and can be calculated from atomic values.⁷⁰ Table XX shows comparisons of calculated and observed boiling points of some halogenated compounds.

TABLE XX

BOILING POINTS CALCULATED FROM ATOMIC VOLUMES

 $V_F = 11.5$ $V_{Cl} = 22.8$ $V_{Br} = 29.1$ $V_I = 39.6$ $V_C = 11$

Compound	V	T_b (calc.)	T_b (observed)
CBr_4	127.4	465°	462°
CCl_3Br	121.1	428	433
CCl_2Br_2	114.8	406	408
CCl_3Br	108.5	375	377
CCl_4	102.2	349	349
CCl_3F	90.4	298	298
CCl_2F_2	78.6	249	248
CBr_3F	109.3	380	380
CBr_2F_2	91.2	300	298
CF_4	55	150	143
CCl_3I	119	419	415
$CClBr=CClBr$	126.8	446	445
$CCl_2=CCl_2$	114.2	392	394

The data of Table XX establish the general reliability of the method of calculation for relatively non-polar compounds. For polar compounds, however, there are appreciable differences between the observed and

⁶⁹ van Arkel, *ibid.*, **51**, 1081 (1932); **52**, 719, 733 (1933); **53**, 91, 246 (1934).

⁷⁰ London, *Z. Physik*, **63**, 245 (1930).

calculated values. For example, the introduction of bromine into benzene to form bromobenzene gives a calculated value some 14° lower than that observed. The discrepancy is due to the polarity of the bromobenzene, and the study of such differences has established relationships between boiling points and dipole moments which permit the calculation of one quantity from the other. Van Arkel has studied these relationships in a large number of aromatic and aliphatic compounds, including 60 sets of isomeric benzene derivatives, and has succeeded in calculating dipole moments from boiling points with satisfactory accuracy. Examples of the agreement between observed and calculated moments are found in Table XXI.

TABLE XXI
DIPOLE MOMENTS CALCULATED FROM BOILING POINTS

Compound	Moment Observed	Moment Calculated	B. P.
<i>o</i> -Dichlorobenzene.....	2.24×10^{-18}	2.72×10^{-18}	178°
<i>m</i> -Dichlorobenzene.....	1.42	1.57	172
<i>p</i> -Dichlorobenzene.....	0	0	171-4
<i>o</i> -Nitrotoluene.....	3.70	3.74	218
<i>m</i> -Nitrotoluene.....	4.20	4.15	230
<i>p</i> -Nitrotoluene.....	4.40	4.38	238

Berger⁷¹ has shown the existence of a close relationship between dipole moments and association of liquids and has been able to predict to some extent the nature of the associated molecules from their moments. The variations with temperature of the dielectric constants and specific refractivities of substances with low dipole moments are attributed to association effects.⁷²

The use of dipole moments in solving problems of molecular structure and also in serving as an empirical tool for purposes of identification has proved exceedingly valuable. It has been found that certain polar groups have characteristic moments which can be considered, as a first approximation, to act independently in determining moments of entire molecules. When more than one polar group is present in a molecule, the resultant molecular moment can be calculated in a direct manner by the vectorial addition of the several individual group moments. In this respect, dipole moments exhibit well-defined additive and constitutive components.

Dipole moments are vector quantities in the sense that the direction of a moment in space is determined by the line which separates the

⁷¹ Berger, *Z. physik. Chem.*, **B22**, 283 (1933).

⁷² Sakurada, *ibid.*, **B24**, 437 (1934).

centers of gravity of the positive and negative charges. When a single polar group is present in a molecule, the direction of the dipole is unimportant for considerations of molecular structure. When several dipoles are present, the directions and magnitudes of each determine the resultant molecular moment. For example, the introduction of Cl into CH_4 to give CH_3Cl produces an electric moment; the introduction of additional chlorine atoms causes a decrease in the moment until a zero moment is reached in CCl_4 . In carbon tetrachloride the four identical moments due to the halogen atoms cancel each other by virtue of their vectorial nature.

A recent very complete compilation of the dipole moments of organic compounds⁷³ illustrates many relationships which have been pointed out by various investigators.

In a series of homologous compounds which have a single polar group, e.g., the *n*-alcohols and *n*-acids, the moments of the members of the series are approximately constant. Thus, the moments for the first 10 normal alcohols are 1.68, 1.69, 1.65, 1.67, 1.63, 1.64, 1.7, 1.67, 1.60, and 1.61. From these values the average moment of the alcoholic C—OH dipole is found to be 1.67. In the fatty acid series, the first member, formic acid, has a greater moment than the higher members but the latter have approximately constant values. The moments of acetic, propionic, and stearic acids have been found to be identical within the experimental error of measurement.⁷⁴ In Table XXII are given the moments of some typical organic series which contain single polar groups; included also are moment values for a few benzene derivatives.

TABLE XXII
MOMENTS OF ORGANIC COMPOUNDS

Compound	Moment	Compound	Moment
<i>n</i> -Hydrocarbons.....	0.0×10^{-18}	Mercaptans.....	1.3×10^{-18}
<i>n</i> -Alcohols.....	1.67	Sulfides.....	1.5
Ethers.....	1.2	Cyanides.....	3.4
Alkyl chlorides.....	2.0	Nitroparaffins.....	3.1
Esters.....	1.8	Nitrites.....	2.2
Primary amines.....	1.3	Nitrates.....	2.9
Secondary amines.....	1.0	Benzene.....	0.0
Tertiary amines.....	0.76	Phenol.....	1.70
Ketones.....	2.7	Anisole.....	1.20
Formic acid.....	1.2	Bromobenzene.....	1.50
<i>n</i> -Acids.....	0.8		

⁷³ *Trans. Faraday Soc.*, **30**, 904 (1934).

⁷⁴ Wilson and Wenzke, *J. Chem. Phys.*, **2**, 546 (1934).

In this table it is seen that the phenols and aromatic ethers show the same moments as the corresponding aliphatic compounds.

The branching of the hydrocarbon chain has a pronounced effect on the moments of aliphatic compounds which have one polar group. In general the moment of the molecule increases as the carbon atom attached to the polar group becomes progressively substituted. Substitution in the chain on a carbon not directly attached to the polar group has little effect on the moment. In Table XXIII are given data for some aliphatic bromides and alcohols.

TABLE XXIII
MOMENTS OF ALIPHATIC BROMIDES AND ALCOHOLS

Compound	Moment	Compound	Moment
<i>n</i> -Butyl bromide	1.88×10^{-18}	<i>n</i> -Amyl alcohol	1.63×10^{-18}
<i>sec.</i> -Butyl bromide	2.12	<i>sec.</i> -Amyl alcohol-(2)	1.66
<i>tert.</i> -Butyl bromide	2.21	<i>sec.</i> -Amyl alcohol-(3)	1.64
Isobutyl bromide	1.97	<i>tert.</i> -Amyl alcohol	1.83
		Isoamyl alcohol	1.81

Sutton⁷⁵ has suggested that the dipole moment of a polar compound as measured may be divided into three components. He expresses this by

$$\mu = m_{\text{primary}} + m_{\text{induced}} + m_{\text{electromeric}}$$

where the three *m* factors represent the primary (permanent) moment, the moment induced on the rest of the molecule, and the moment due to the electromerism or conjugation of rings.

Sutton has calculated the moments induced by a polar group on the carbon atoms of aliphatic saturated and conjugated unsaturated chains. The results of these calculations are illustrated in Fig. 9; in this figure are shown the relative moments induced on the carbon atoms successively removed from the polar group.

The moments of molecules with more than one polar group are governed by the degrees of polarity and the positions of these polar groups in the molecules. Groups with similar polar properties when placed near each other tend to increase the moment over that found when a single dipole is present. On the other hand when two groups of opposite polar character (such as NO₂ and NH₂) are situated close to each other in a molecule, the effect is to neutralize the moments. When such dissimilar

⁷⁵ Sutton, *Proc. Roy. Soc. (London)*, **A133**, 668 (1931).

groups are placed at opposite ends of a non-polar chain, the resultant moment increases with the distance of separation of the groups. Although the non-polar chain has very little influence on the resultant molecular moment, the center or non-polar nucleus does determine the direction of polar forces and must be considered in any attempt to cal-

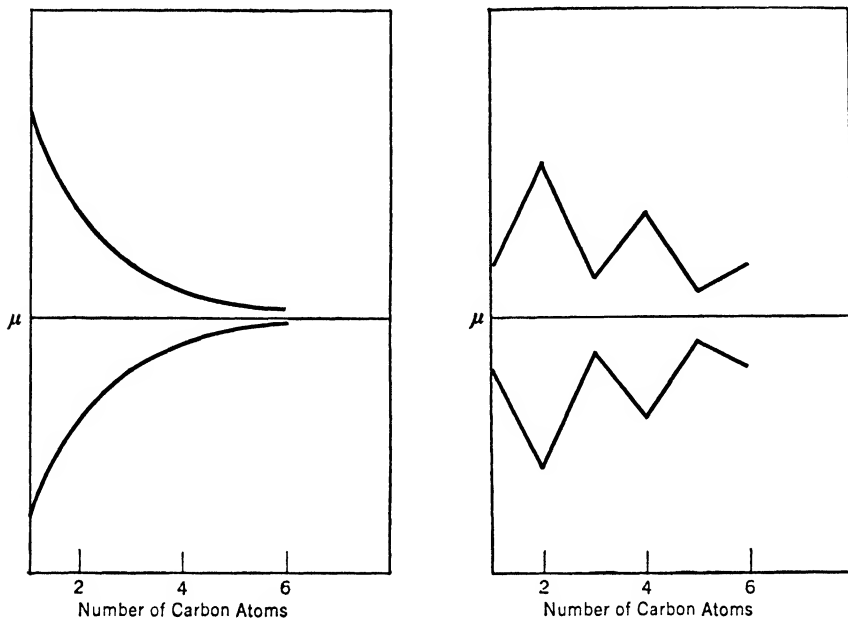


FIG. 9 *

Relative influence of increasing the number of carbon atoms on the moment.

Relative influence of increasing the length of a conjugated chain on the moment.

culate dipole moments resulting from the joint action of several polar groups.

As a means of calculating dipole moments for complex molecules, bond and group moments have been assigned to various structural units. The resultant moments of such complex molecules are obtained by vector addition of the individual group moments. If two dipoles are located at opposite ends of a molecule, vector addition becomes simple algebraic addition; but if the groups are oriented at an angle other than 180° to each other, it is necessary to calculate the resultant moment from the cosine law. This latter case is best illustrated in the benzene series, where groups are fixed in the *ortho*, *meta*, or *para* positions with respect to each other. Fig. 10 is a graphical representation of the addition of

* From Sutton, *loc. cit.* (Courtesy of publishers.)

the vector dipoles μ_1 and μ_2 . The moments μ_o , μ_m , and μ_p are the resultant molecular moments when the groups of moment μ_1 and μ_2 , are in the *ortho*, *meta*, and *para* positions, respectively.

Group moments are vectorially added by use of the equation,

$$\mu = \sqrt{\mu_1^2 + \mu_2^2 + 2\mu_1\mu_2 \cos \theta}$$

where μ_1 and μ_2 are the moments of the groups and θ is the angle of separation of the dipoles having these moments. In benzene deriva-

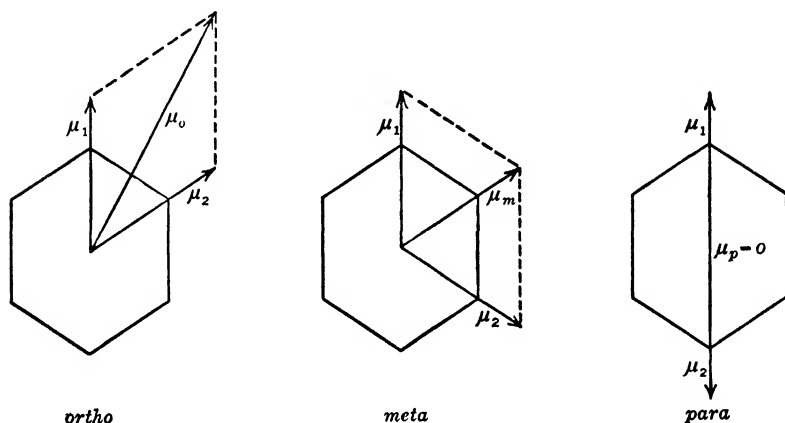


FIG. 10.—Representation of dipole forces in disubstituted benzene derivatives.

tives, θ becomes 60° for the *ortho*, 120° for the *meta*, and 180° for the *para* configurations. If μ_1 and μ_2 are identical, the resultant moments of *ortho*, *meta*, and *para* configurations become $\mu\sqrt{3}$, μ , and 0, respectively.

In Table XXIV are given moment values for a variety of atoms and groups; these values have been used by Sutton⁷⁵ to calculate the resultant dipole moments of molecules which contain one or more polar groups. Separate values are given to be applied in calculating moments of aliphatic and aromatic compounds, and several moments are given for application to olefinic derivatives. Sutton has estimated his electromeric effect to derive values given in the column headed m_e ; the sign of the values in this column indicates the orienting nature of the group, i.e., a positive m_e means an *ortho-para* directing group while a negative m_e signifies a *meta* directing group (p. 140). The magnitude of m_e is a relative measure of the orienting strength of the group.

In Table XXV are shown some observed molecular moments and the values calculated by the method of vector addition of individual atomic and group moments. The agreement is generally good, the great-

est discrepancies occurring in molecules such as the nitro-iodo-benzenes which have two strongly polar dissimilar groups.

TABLE XXIV
COMPONENT FACTORS FOR CALCULATION OF MOMENTS (Sutton)

Group	m_{aromatic}	$m_{\text{aliphatic}}$	m_e	m_{ethylene}
—CH ₃	+0.45	0.0	+0.45
=O.....	-1.06	-1.29	+0.23
—NH ₂	+1.55	+1.23	+0.32
—Cl.....	-1.56	-2.15	+0.59	-1.66
—Br.....	-1.52	-2.21	+0.69	-1.48
—CH ₂ Cl.....	-1.82	-2.03	+0.21	-2.00
—I.....	-1.27	-2.13	+0.88
—OH.....	-1.7	-1.83	+0.15
—OCH ₃	-1.2	-1.16	0
—CHO.....	-2.75	-2.46	-0.29
—COCH ₃	-2.97	-2.79	-0.18
—CO ₂ CH ₃	-1.93	-1.71	-0.22
=C=O.....	-3.04	-2.76	-0.28
—C≡N.....	-3.89	-3.46	-0.43
—NO ₂	-3.93	-3.05	-0.88

TABLE XXV
OBSERVED AND CALCULATED MOMENTS

Compound	Observed	Calculated	Compound	Observed	Calculated
Acetaldehyde	2.7	2.7	Toluene	0.4	0.4
<i>trans</i> -CHCl=CHCl	0	0	Nitrobenzene	3.93	3.93
<i>cis</i> -CHCl=CHCl	1.9	1.9	Iodobenzene	1.30	1.30
Allyl chloride	1.97	1.90	<i>o</i> -Nitrotoluene	3.66	3.75
Vinyl bromide	1.48	1.50	<i>m</i> -Nitrotoluene	4.14	4.15
<i>o</i> -Xylene	1.68	1.70	<i>p</i> -Nitrotoluene	4.42	4.43
<i>o</i> -Iodotoluene	1.21	1.15	<i>o</i> -Nitroiodobenzene	3.66	4.72
<i>m</i> -Iodotoluene	1.57	1.54	<i>m</i> -Nitroiodobenzene	3.22	3.47
<i>p</i> -Iodotoluene	1.71	1.70	<i>p</i> -Nitroiodobenzene	2.63	2.63

As an alternate method of computing molecular dipole moments, it has been suggested that additive values should be assigned to linkages rather than to individual atoms.⁷⁶ Examples of these moment com-

⁷⁶ (a) Eucken and Meyer, *Physik. Z.*, **30**, 397 (1929). (b) Fuchs and Donle, *Z. physik. Chem.*, **B22**, 18 (1933). (c) Allard, *Compt. rend.*, **192**, 1455 (1931).

ponents for separate linkages, together with some computed molecular moments derived from them by vector addition, are given in Table XXVI. The agreement between observed and calculated moments as

TABLE XXVI
(A) LINKAGE MOMENTS

Linkage	Moment	Linkage	Moment	Linkage	Moment
C—C	0.0	C=O	2.79	C—O—	1.1
C—H	0.4	O—H	1.63	C—Cl	1.5
C—Br	1.5	C—CH ₃	0.4	C—I	1.3

(B) MOLECULAR MOMENTS CALCULATED FROM LINKAGE VALUES

Compound	Observed	Calculated	Compound	Observed	Calculated
<i>o</i> -Xylene	0.68	0.70	<i>p</i> -Chlorophenol	2.3	2.4
Allyl bromide	1.93	1.90	Methyl alcohol	1.68	1.55
Ethyl chloride	1.98	1.9	<i>p</i> -Cresol	1.6	1.5

shown in this table is good. It will be necessary to extend the calculation to numerous other compounds before a decision can be made as to the relative merits of the method of linkage moments as compared to that of group moments.

Many general observations have been made. Wolf and Gross⁷⁷ have shown that there exists the same type of alternation in the moments of adjacent members of homologous series as is shown in melting points, specific heat, etc. Smyth⁷⁸ has interpreted some of his data to mean that long-chain molecules are fairly rigid in structure; this investigator has demonstrated that normal and *iso* aliphatic compounds containing a polar group show the same moments if the branching occurs at least two atoms from the polar group. It has been found⁷⁹ that if a carbon chain contains unsaturated conjugated linkages the dipole effect of a polar group attached to the chain is transmitted along the chain.

The use of dipole-moment data in attacking problems of structural organic chemistry has been fruitful in a number of cases. From the

⁷⁷ Wolf and Gross, *Z. physik. Chem.*, **B14**, 305 (1931).

⁷⁸ Smyth and Walls, *J. Am. Chem. Soc.*, **53**, 527 (1931); **54**, 2261 (1932).

⁷⁹ Farmer and Warren, *J. Chem. Soc.*, 1302 (1933).

knowledge of individual group moments and the determination of molecular moments it has been possible to calculate the valence angles of some atoms like oxygen and sulfur.⁸⁰ Certain *para*-disubstituted benzene derivatives such as the diethyl ether of hydroquinone and the dimethyl ester of terephthalic acid have been found to be non-coplanar inasmuch as they exhibit definite dipole moments.⁸¹ The structures of a number of *cis-trans* isomers have been definitely established because of the fact that *cis* forms have appreciable moments whereas *trans* forms show much smaller or zero moments (p. 374). The fact that tetranitromethane has zero moment argues strongly that all four nitro groups are identical and are located at the corners of a tetrahedron; on the basis of chemical evidence it had been concluded that one of the NO₂ groups assumed a nitrite structure. Dipole-moment measurements of a number of tetrasubstituted methanes show that in none is it necessary to assume a pyramidal structure for such molecules as has sometimes been postulated.

Wolf⁸² has attacked the problem of restriction of rotation about the C—C single bond in optical isomers by means of dipole measurements. If free rotation exists, the moments of *d*, of *l*, and of *meso* forms of enantiomorphs should be identical; if free rotation does not exist, the moments of the *d* and *l* forms should be identical and should be different from that of the *meso* form. Wolf found that the dipole moments of pure diethyl *d*-tartrate and diethyl *l*-tartrate were the same, 3.12×10^{-18} e.s.u., while the moment of diethyl *meso*-tartrate is higher, 3.66×10^{-18} e.s.u. The measurements therefore offer strong support for the classical theories of molecular structure of LeBel and van't Hoff (p. 158).

The number of applications of the study of dipole moments to problems of molecular structure is large and the results are very encouraging. Although the interpretation of data is sometimes ambiguous, the general value of the method is well established. From the standpoint of the relationship between structure and physical properties, the dipole moment affords an excellent example of a property which exhibits additive and constitutive components.

The discussion of dipole moments logically leads to the consideration of other properties from which may be determined intramolecular dimensions and configurations.

⁸⁰ Glasstone, "Recent Advances in Physical Chemistry," 2nd ed., Blakiston's Son and Co., Philadelphia (1933), p. 135.

⁸¹ Debye, "Polar Molecules," Chemical Catalog Co., New York (1929).

⁸² Wolf, *Trans. Faraday Soc.*, **26**, 315 (1930).

The Structures of Organic Compounds as Determined from X-ray Diffraction Measurements

The study of the arrangement of atoms in crystals of inorganic compounds has been successfully attacked by the use of x-rays. By studying the diffraction of x-rays from crystalline surfaces it has been possible to calculate the distances between the planes of atoms in the crystals just as it is possible to calculate the distance between rulings of a grating from light-diffraction measurements. The success of the x-ray diffraction method for determining the structure of a crystal depends on the degree of symmetry of its internal structure; in general, the more symmetrically arranged structural units are the more easily analyzed.

The applicability of the x-ray diffraction method for determining the molecular configurations of organic compounds has been somewhat limited because most organic substances are quite complex from a structural viewpoint and consequently do not exhibit high degrees of symmetry. Nevertheless, a great deal of valuable information has been obtained about the structures of very fundamental types of compounds. It must be pointed out that the investigator of the molecular structures of organic compounds has had considerable help in his work from the classical pictures of such compounds as postulated for many years by organic chemists; he has had certain clues as to the direction he should take in the analysis of his data.

The results which have been obtained from the study of the structures of organic compounds by x-ray analysis have been summarized in a number of publications.^{83, 84, 85, 86}

The structures of a number of halogenated aliphatic hydrocarbons have been determined with some degree of certainty. Iodoform, 1,2,3,4,5,6-hexabromo- and 1,2,3,4,5,6-hexachlorocyclohexane, hexabromoethane, *cis* and *trans* dichloroethylene, and 1,1- and 1,2-dichloroethane have thus been investigated. It was found that in all of them the structures which fit the data best show carbon to have valence angles corresponding to tetrahedral angles as postulated by the organic chemist. The cyclohexane derivatives have structures which place the carbon atoms on a puckered ring (p. 5). The distance between chlorine atoms in *cis*-dichloroethylene is 3.6 Å as compared to 4.1 Å for *trans*-dichloroethylene (p. 375).

⁸³ Hendricks, *Chem. Rev.*, **7**, 431 (1930).

⁸⁴ Bragg and Bragg, "The Crystalline State," Vol. I, Bell (1934).

⁸⁵ Bernal and Crowfoot, "Ann. Repts. Chem. Soc. (London)," Vol. XXX, p. 379 (1933).

⁸⁶ Robertson, *Chem. Rev.*, **16**, 417 (1935).

In the diamond crystal each carbon atom is surrounded by four others at a distance of 1.54 \AA placed at the corners of a tetrahedron. In graphite the carbon atoms in one plane form a series of interlocking hexagons, the distance from carbon to carbon being 1.42 \AA .

It is very interesting that the structure of hexamethylenetetramine has been accurately determined and is identical with one accepted by organic chemists.

A number of aliphatic compounds has been investigated, a few in detail. Of the *n*-hydrocarbons, nonacosane ($\text{C}_{29}\text{H}_{60}$), has been carefully analyzed. It was found that the carbon chain is continuous and does not fold back on itself. The carbon atoms lie on a plane and have the zigzag structure demanded by the tetrahedral carbon atom. In the zigzag structure the distance between alternate carbon atoms was found to be 2.54 \AA , and the C—C distance is very near 1.54 \AA which was found for diamond. Other hydrocarbons show an increase of 2.54 \AA for every two CH_2 groups added to the molecule.

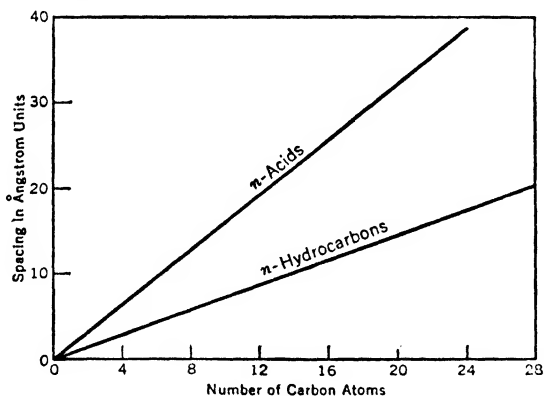


Fig. 11.*—Regular increase in chain length of aliphatic homologs.

The gross structures of the *n*-aliphatic acids,⁸⁷ the *n*-aliphatic alcohols,⁸⁸ and the *n*-aliphatic dicarboxylic acids have been investigated. In all these series, the carbon atoms lie on a plane and have a zigzag arrangement. In both the acid and alcohol series, it was found that the addition of each successive CH_2 group caused an increase in the unit structure twice as great as that observed for the *n*-hydrocarbons. This is shown in Fig. 11, which is taken from tabulated data.⁸⁹ The

* From Ewald and Harmann, *loc. cit.* (Courtesy of publishers.)

⁸⁷ Francis, Piper and Malkin, *Proc. Roy. Soc.*, (London), **A128**, 214 (1930).

⁸⁸ Wilson and Ott, *J. Chem. Phys.* **2**, 231 (1934).

⁸⁹ Ewald and Harmann, "Strukturbericht, 1913-28," Akad. Verlag., Leipzig (1931), p. 684.

facts just cited argue that there are two molecules per unit structure for the alcohols and acids. In the fatty acid series the unit spacings for the even carbon compounds are nearly equal to the spacings of the next higher odd carbon compounds. The addition of two CH_2 groups to an even member of the dicarboxylic acid series causes an increase in unit spacing equal to that for the hydrocarbons; the addition of CH_2 groups to an odd member produces a change twice as great as that for the hydrocarbons.

Müller⁹⁰ has discussed the effect of the zigzag structure of the hydrocarbon chain upon the properties of the even and odd members of the dicarboxylic acid series. He has concluded that the alternating properties of this series can be explained by their structures. It can be seen from the arrangement of the zigzag chain for the hydrocarbons, *n*-alcohols, or *n*-acids that the symmetry properties of the odd and even members of the series will be different. This difference may well account for the alternating properties observed for these compounds.

The structures of a number of aromatic compounds (pp. 52, 267) have been very accurately determined. The analysis of x-ray diffraction data for anthracene, naphthalene, *sym*.-tetramethylbenzene, benzoquinone, and bibenzyl has led to unambiguous structures for these compounds. Naphthalene and anthracene are composed of plane, regular hexagons in which the carbon-carbon distance is 1.41 Å. This figure is that observed for the C—C distance in graphite. In *sym*.-tetramethylbenzene the carbons in the ring form a regular, plane hexagon with a C—C distance of 1.41 Å; the methyl carbon atom is 1.47 Å removed from its adjacent ring carbon atom. The methyl groups lie in the plane of the ring and are slightly displaced from the positions demanded by the hexagonal ring, the displacement being 3° greater than that for a symmetrical structure.

The structure of benzoquinone is that proposed by the organic chemist except that the ring is slightly skew. An interesting point in connection with the benzoquinone structure is that the carbons joined by a double bond are 1.32 Å apart while the singly bonded carbons are removed 1.50 Å from each other. The carbon-oxygen distance is 1.14 Å.

The results of the analysis of the structure of bibenzyl show that the two benzene rings are of the regular plane hexagon type; they do not lie on one plane but are parallel to each other and are on opposite sides of the two CH_2 groups. The carbon atoms in the CH_2 groups exhibit tetrahedral bond angles. The CH_2 groups are situated at 1.47 Å from the benzene rings and are removed from each other by 1.58 Å.

⁹⁰ Müller, *Proc. Roy. Soc.*, (London), **A124**, 317 (1929).

The structures of a number of other aromatic compounds have been less accurately determined. All these structures are consistent with those found for the more carefully investigated compounds.

In Table XXVII are collected some of the interatomic distances determined from x-ray diffraction data.

TABLE XXVII
INTERATOMIC DISTANCES IN SOME ORGANIC COMPOUNDS

Compound	Distance in Å	Compound	Distance in Å
Diamond	C—C 1.54	Hexamethylbenzene .	C—C aromatic 1.42
Graphite	C—C 1.42		C—CH ₃ 1.48
1,2,3,4,5,6-Hexabromo- cyclohexane	C—Br 1.94	Nonacosane	C—C 1.54
Hexabromoethane	C—Br 1.97	Hydrocarbons	C—C 1.54
1,2,3,4,5,6-Hexachloro- cyclohexane	C—Cl 1.81	Anthracene	C—C 1.41
Hexachloroethane	C—Cl 1.81	Naphthalene	C—C 1.41
Urea	C—N 1.37	Tetramethylbenzene .	C—C aromatic 1.41
	C=O 1.25		C—CH ₃ 1.47
Thiourea	C—N 1.35	Bibenzyl	C—C aromatic 1.41
	C=S 1.64		C—C aliphatic 1.58
Hexamethylenetetra- mine	C—N 1.42		C—CH ₂ aromatic- aliphatic 1.47
		Benzoquinone	C—C in ring 1.50
			C=C in ring 1.32
			C=O 1.14
		<i>p</i> -Diphenylbenzene . . .	C—C aromatic 1.42
			C—C between rings 1.48

The study of x-ray diffraction data has proved very valuable in elucidating the gross structures of certain high-molecular-weight compounds.⁹¹ Such data have helped establish the generally accepted structure of cellulose. In cellulose (p. 1586) it was found that bundles of very long, parallel, chain molecules constitute the fibers of ramie, cotton, etc. The chains are composed of unit structures, presumably cellobiose units, which are linked together through oxygen atoms. The length of two units is 10.3 Å, and the distance between two parallel chains is 8.3 Å in one direction and 7.9 Å in the other.

⁹¹ Randall, "The Diffraction of X-rays and Electrons by Amorphous Solids, Liquids, and Gases," John Wiley and Sons, New York (1934), p. 200.

It has been shown by x-ray experiments that rubber in a stretched condition is much more highly oriented than in the unstretched state. There is still controversy over the actual structure of rubber, but it is generally accepted that the material consists of long molecular chains of isoprene units, each unit being 4.05 Å in length.

Data similar to those on rubber and cellulose have been obtained for hair, wool, natural silk, and proteins. Owing to the lack of exact chemical knowledge as to the constitution of these substances, no very definite structures have been assigned.

Electron Diffraction by Organic Compounds

It has been discovered that moving electrons exhibit properties characteristic of wave motion and that the reflection or diffraction of electrons by solids, liquids, or gases obeys certain laws which apply to the action of light or x-rays. Advantage has been taken of this fact in determining molecular structures by a method similar to that used in x-ray diffraction experiments. It is usually experimentally simpler to work with gases than solids and liquids in electron diffraction work, and most of the data on the structures of compounds have been obtained when working with the gaseous state.

The electron diffraction method of determining the structures of substances has a number of advantages over the x-ray method. When measurements are made on gases, complications due to the symmetry properties of intermolecular orientation are lacking and the data are generally more easily interpreted. Furthermore, the electron diffraction method is capable of showing the locations of atoms of lower atomic number than those which can be placed by x-ray data. This latter fact is of great importance to the organic chemist since carbon has a relatively low atomic number and can be located in a molecule by the electron method even though much heavier atoms are present.

The work on the use of electron diffraction measurements to determine molecular structures was only recently initiated (1930), but much valuable information has been obtained. The results have been conveniently summarized by Randall.⁹¹

Definite evidence for the planar structure of benzene and the puckered ring structure of cyclohexane has been obtained from electron diffraction data. The structures of a few compounds have been determined by both x-ray and electron diffraction methods, and the agreement of the two results is entirely satisfactory. This is shown in Table XXVIII.

A few of the results of electron diffraction studies of organic compounds are summarized in Table XXIX.

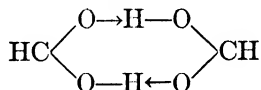
TABLE XXVIII
DISTANCES BETWEEN IODINE ATOMS DETERMINED BY X-RAY AND ELECTRON
DIFFRACTION MEASUREMENTS

Substance	Electron Diffraction by Vapor	X-ray Diffraction by Crystal
Iodine	2.64 Å	2.70 Å
1,4-Diiodobenzene	6.85	6.85
1,3-Diiodobenzene	5.97	5.92

TABLE XXIX
DISTANCES BETWEEN ATOMS DETERMINED BY ELECTRON
DIFFRACTION MEASUREMENTS

Substance	Interatomic Distance in Å	Remarks
Ethane	C—C 1.52	Plane structure; CH angle 110°
Ethylene	C=C 1.30	
Acetylene	C≡C 1.22	
Diacetylene	C—C 1.51	Tetrahedral angle
	C≡C 1.20	
Propane	C—C 1.52	
Cyclopentane	C—C 1.51	Tetrahedral angle
<i>trans</i> -Dichloroethylene	Cl—Cl 4.33	
<i>cis</i> -Dichloroethylene	Cl—Cl 3.30	
Methyl bromide	C—Br 2.06	I—C—I angle 125°
Methyl iodide	C—I 2.28	
Methylene iodide	C—I 2.28	
	I—I 4.06	Linear structure
Carbonyl sulfide	C=S 1.58	
	C=O 1.13	
Bromoform	C—Br 2.05	Br—C—Br angle 115°
	Br—Br 3.46	
Phosgene	C=O 1.12	
	C—Cl 1.80	Cl—C—Cl angle 110°
	Cl—O 2.62	
Acetyl chloride	C=O 1.14	
	C—C 1.54	C—C—Cl angle 110°
	C—Cl 1.82	
Methyl azide	N≡N 1.26	
	N=N 1.10	C—N—N angle 150° —N=N≡N linear
	C—N 1.47	
Carbon dioxide	O—O 2.2	Linear structure
Carbon disulfide	S—S 3.0	Linear structure
Carbon tetrachloride	C—Cl 1.76	Tetrahedral structure
Benzene	C—C 1.39	Plane hexagonal structure

An interesting result of electron diffraction measurements on the vapor of formic acid has been obtained by Pauling and Brockway.⁹² They found that two molecules of formic acid are joined together by hydrogen bonds to form the symmetrical structure,



in which the distance between oxygen atoms in the O—H←O system is 2.67 Å and the C—O distance 1.29 Å. The O—C—O angle is 125°. This study provides very definite proof of the existence of chelate ring formation in this compound.

Pauling⁹³ and Pauling and Huggins⁹⁴ have prepared a set of atomic radii to be used in calculating the interatomic distance in covalent bonds. A few of the values are: single bonds, H = 0.29, C = 0.77, N = 0.70, O = 0.66, F = 0.64, Cl = 0.99, Br = 1.14, I = 1.33, Si = 1.17, P = 1.10, S = 1.04, Ge = 1.22, As = 1.21, Se = 1.17, Sn = 1.40, Sb = 1.41, and Te = 1.37 Å; double bonds, C = 0.69, N = 0.63, O = 0.59, and S = 0.94 Å; triple bonds, C = 0.61 and N = 0.55 Å. By the addition of these atomic radius values, the interatomic distances in a number of compounds have been calculated and found to agree with observed values.

An example of this agreement is shown in a study by Brockway and Jenkins⁹⁵ of the structures of some metallic and non-metallic alkyls as determined by electron diffraction experiments. In Table XXX are presented the results of their determinations together with the values calculated assuming additivity of atomic radii.

TABLE XXX
BOND DISTANCES AND RADIUS SUMS IN METHYL COMPOUNDS

Bond	Experimental Value	Radius Sum	Bond	Experimental Value	Radius Sum
C—C	1.55	1.54	S—C	1.82	1.81
N—C	1.47	1.47	Cl—C	1.77	1.76
O—C	1.42	1.43	Ge—C	1.98	1.99
F—C	1.42	1.41	Br—C	1.91	1.91
Si—C	1.93	1.94	Sn—C	2.18	2.17

⁹² Pauling and Brockway, *Proc. Natl. Acad. Sci. U. S.*, **20**, 336 (1934).

⁹³ Pauling, *ibid.*, **18**, 293 (1932).

⁹⁴ Pauling and Huggins, *Z. Krist.*, **87**, 205 (1934).

⁹⁵ Brockway and Jenkins, *J. Am. Chem. Soc.*, **58**, 2036 (1936).

Absorption Spectrum and Raman Effect

It will be profitable to discuss briefly the underlying principles which govern the absorption of light by molecules. A simple diatomic molecule may be considered to have a dumbbell structure in which the two ends which are the atoms vibrate very rapidly with respect to each other along the line which separates them. In addition to this constant rapid vibration, the molecule slowly rotates as a whole about its center of gravity. The electronic structure of the molecule in its normal, unexcited state remains constant during the vibrations and rotation. When energy in the form of light is absorbed by the molecule one or all of three changes may occur; the electronic structure of the molecule may be altered, the amplitude of vibration of the atoms with respect to each other may be increased, or the frequency of rotation of the molecule may undergo a change.

When the light absorbed by a molecule results in a change in its electronic structure, the molecule is said to be electronically excited; when the absorbed light causes a change in the amplitude of vibration of the atoms with respect to each other, the molecule is vibrationally excited; when the absorbed light changes the rate of rotation of the molecule, the molecule is rotationally excited. Usually the absorption of light by a molecule results in simultaneous changes in its electronic, vibrational, and rotational states; and in the analysis of the spectral absorption data it is necessary to separate the absorbed light into the three components which contribute to the three types of excitation.

Changes in the electronic structures of molecules require relatively large amounts of energy, and the absorption of light in the visible or ultra-violet spectral regions is therefore usually associated with an electronic change. The amount of energy necessary to produce vibrational or rotational excitation is comparatively small, and the absorption of infra-red light consequently indicates that the excitation produced is of one or both of these types. It must be repeated that with the absorption of ultra-violet and visible light not only does electronic excitation occur but also simultaneous rotational and vibrational excitations; with the absorption of infra-red light simultaneous vibrational and rotational excitations usually take place.

In the Raman effect, light of a given wave length is absorbed by a molecule and a certain fraction of its energy is used in producing a purely vibrational excitation. The energy not used in this excitation is liberated from the molecule as light of a wave length longer than that of the exciting source. The difference in wave lengths (or frequencies) of the original and emitted light is therefore a measure of the excitation

of the vibrating system. Since the Raman effect is measured by differences, the result obtained is independent (within certain limits) of the frequency of the exciting light. Furthermore, since infra-red absorption data also serve as a measure of vibrational energy effects, data obtained from the Raman effect and from infra-red absorption should be comparable; this is found to be true for a number of substances.

The preceding discussion has been confined to diatomic molecules. The problem of the analysis of spectroscopic data for polyatomic molecules becomes exceedingly complicated owing to the large number of vibrating systems and to the increased possibilities for electronic transitions. Indeed, only for polyatomic molecules with high degrees of symmetry have the data been successfully analyzed. Nevertheless, since each vibrating system of atoms has a characteristic frequency of oscillation, it has been possible to assign certain fundamental frequencies to the various groups of atoms which occur in organic compounds. Since infra-red absorption and Raman-effect measurements are readily interpreted because they do not involve electronic changes, these measurements have been extensively undertaken in the determination of characteristic group vibration frequencies. It is recognized and appreciated that constitutional factors have definite effects on the frequencies which the various vibrating atomic couplets exhibit, but the variations due to constitution are usually not too great to impair seriously the assignment of the fundamental frequencies to particular pairs of atoms.

Raman Effect. Since the study of the Raman effect affords a direct, theoretically simple way of determining the fundamental frequencies of vibration characteristic of various atomic linkages, considerable experimental work has been done in the measurement of the Raman effect in organic molecules. Hibben has given an exhaustive summary of the experimental data.⁹⁶

In presenting data on the Raman effect, the usual method of expressing the magnitude of the difference in frequencies of the exciting and emitted light is in terms of reciprocal centimeters, cm.^{-1} , i.e., the number of waves per centimeter. The values of these differences are termed the Raman shifts. From these Raman shifts it is possible to calculate by simple mechanical principles the forces binding the atoms of the vibrating system. In Table XXXI are given a few of these forces for different types of linkages. It will be seen that the forces for single, double, and triple bonds are surprisingly constant irrespective of the atoms which are bound together. Although the binding forces remain approximately constant for one type of bond, the Raman shifts increase as the weights of the atoms in the vibrating couplets decrease.

⁹⁶ Hibben, *Chem. Rev.*, **18**, 1 (1936).

TABLE XXXI

VALENCE FORCES (F) FOR DIFFERENT TYPES OF LINKAGE

Linkage	$F \times 10^{-6}$ Dynes cm. ⁻¹	Linkage	$F \times 10^{-6}$ Dynes cm. ⁻¹
H—H.....	5.38	N—H.....	6.39
C—H.....	5.02	O—H.....	6.72
C—C.....	4.40	C—N.....	4.53
C—O.....	5.05	C≡C.....	14.82
C=C.....	11.0	C≡N.....	19.23
C=O.....	11.7	C=O.....	18.83
O=O.....	11.4		

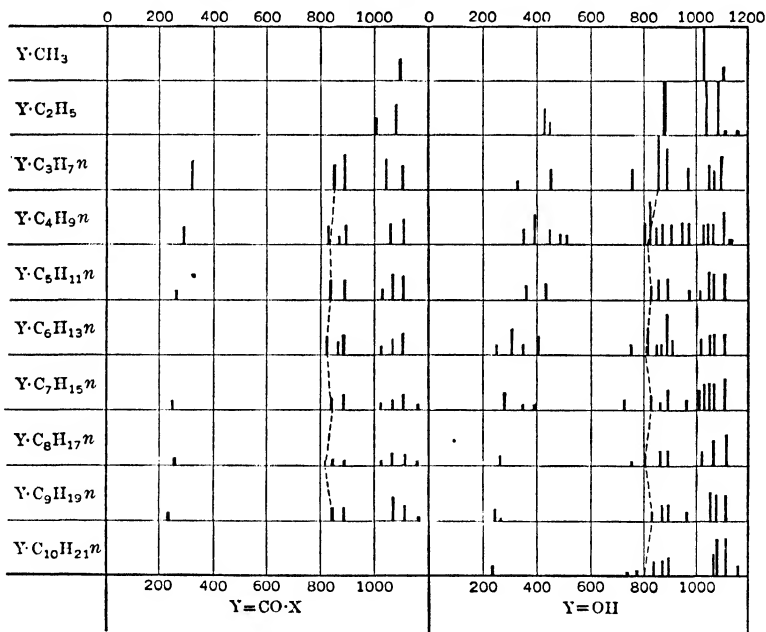


FIG. 12.—Graphical representation of Raman shifts for organic compounds.

A convenient method of presenting Raman shift data is illustrated in Fig. 12, where the magnitude of the shift is represented as a line placed in its proper graphical position.

The use of Raman data, aside from their theoretical importance, is exceedingly valuable in the identification of certain types of linkages in organic compounds. The fact that the various types of bonds and

atomic couplets exhibit characteristic Raman shifts which can be observed independently of the environment of the compound and hence which are free of intermolecular complications makes the method one of great practical importance for structure determination. It is true that constitutive factors affect the absolute values of the shift characteristic of any vibrating atomic group, but usually the perturbations are not great enough to prevent identification of the particular bond or group producing the shift. The effect of constitution on the Raman shifts characteristic of the C—H linkage is shown in Table XXXII.

TABLE XXXII
RAMAN SHIFTS FOR C—H VIBRATIONS

Linkage		Linkage	
C—H aliphatic.....	2918	C—H aromatic.....	3054
H		H	
C—C—H.....	{ 2930 2862	C—C—C.....	2970
H		H	

In Table XXXIII are collected values of the Raman shifts for a few characteristic atomic linkages. The figures represent either the ranges of values observed or rough averages since as shown in Table XXXII the absolute numerical values depend to some extent on the structures of the molecules investigated.

TABLE XXXIII
RAMAN SHIFTS FOR CHARACTERISTIC LINKAGES

Linkage	Shift, cm. ⁻¹	Linkage	Shift, cm. ⁻¹
C—H aliphatic.....	2920-2970	S—H.....	2570
C—H aromatic.....	3054	C—S.....	645
C—C aromatic.....	1580-1608	N=O nitrate.....	1640
C—C aliphatic.....	800-860	nitrite.....	1640
C=C.....	1600-1650	nitro.....	1565
C≡C.....	2100-2250	C=N.....	1650
C—OH.....	820-880	C≡N.....	2150
O—H.....	3400	C—N nitro.....	910-930
C=O acid.....	1654	amine.....	880
ketone.....	1710	N—O.....	1000-1080
aldehyde.....	1720	C—Cl.....	650-710
ester.....	1720	C—Br.....	570-600
anhydride.....	1750	C—I.....	500-530
C—C.....	1188 and 867		
\ /			
C			

The effect of substitution on the Raman shift of the C—C aromatic linkage of benzene has been investigated. Representative data are presented in Table XXXIV.

TABLE XXXIV

RAMAN SHIFTS FOR THE C—C LINKAGE IN SUBSTITUTED BENZENES

Substance	Positions of Substituent Groups	Shift, cm. ⁻¹
C ₆ H ₆ Cl	1580
C ₆ H ₄ Cl ₂	1,2	1572
	1,3	1572
	1,4	1572
C ₆ H ₃ Cl ₃	1,2,4.....	1564
	1,3,5.....	1563
	1,2,3.....	1554
C ₆ H ₂ Cl ₄	1,2,4,5.....	1563
	1,2,3,5.....	1558
	1,2,3,4.....	1552
C ₆ HCl ₅	1,2,3,4,5.....	1553
C ₆ Cl ₆	1,2,3,4,5,6.....	1503

Kohlrausch⁹⁷ has observed some alternation of the Raman shifts for the odd and even members of certain paraffin series. His results are presented in Fig. 12.

The value of Raman effect data in determining structures or in differentiating between proposed structures can be illustrated by several of the many problems investigated.

It has been found that the Raman shift due to the C=C bond in *cis-trans* isomers is uniformly greater by at least 15 units for the *trans* compounds than for the *cis* compounds (p. 375); the importance of this generalization is obvious. By a careful study of the effect on the characteristic C=C shift of substitution on the carbons of the ethylenic linkage, the structures of rhodinol and citronellal have been determined.

It has been found that the characteristic C=O shift of formaldehyde disappears when this compound is added to water; on solution the Raman spectrum becomes similar to that of glycol. These facts are interpreted to mean that the reaction $\text{CH}_2\text{O} + \text{H}_2\text{O} \rightarrow \text{CH}_2(\text{OH})_2$ occurs. The fact that no C=O shifts are found in paraldehyde or paraformaldehyde is positive evidence that these polymers are cyclic in structure.

In an equilibrium mixture of a tautomeric substance such as acetoacetic ester, both C=C and C=O shifts are observed. When the possibility of tautomerism is removed by dialkylation of the central carbon atom, the C=C shift is no longer observable. These facts

⁹⁷ Kohlrausch, *Z. physik. Chem.*, **B24**, 380 (1934).

afford confirmatory evidence for the classical structure of these compounds proposed by the organic chemist.

The presence in the oximes of a Raman shift which corresponds to that of the $C=N$ linkage must be definitely considered in any proposal of a structure for these compounds. Similarly, it has been established from measurements of the Raman effect in nitriles and isonitriles (p. 705) that these compounds contain a $C\equiv N$ linkage.

At the present time, a vast amount of work is being carried out in which the Raman shifts of organic compounds are being measured. The usefulness of these data for purposes such as structure and entropy determinations is unquestioned, but caution must be observed in interpreting them. Without the proper appreciation of the limitations of the method and of the necessity for observing certain experimental precautions in determining the shifts, a great amount of conflicting data can be accumulated. In the main the method has great value for certain purposes.

Infra-red Absorption Spectra. The systematic study of infra-red absorption spectra necessitates the use of an experimental technique and equipment not readily available to the average research worker. For this reason careful systematic studies of the relationships between chemical structure and infra-red absorption measurements are not numerous. As mentioned above, for the purpose of structural identification the examination of the Raman shifts in organic compounds often provides equivalent data.

One recent investigation of infra-red absorption measurements has led to some very interesting conclusions.⁹⁸ It has been observed that the OH group in alcoholic and phenolic compounds is responsible for an absorption band in the neighborhood of $15,000 \text{ \AA}$. This fact was used in the investigation of chelate ring (or hydrogen bond) (p. 1638) formation. The infra-red absorption spectra of a number of compounds in which such bond formation was thought configurationally possible were studied. The absence of the $15,000 \text{ \AA}$ band was considered proof that the OH group as such was not present in the molecule and that chelation had occurred. By means of the measurements a large number of compounds was found to undergo ring formation. The following generalizations were made regarding the factors favorable to hydrogen bond formation:

1. The $O-H\cdots O$ distance should be about 2.60 \AA .
2. The ring should be free of strain.
3. There should be a limited number of bonds in the ring about which there can be free rotation.

⁹⁸ Hilbert, Wulf, Hendricks, and Liddel, *J. Am. Chem. Soc.*, **58**, 548 (1936).

It was found for every compound but one that chelation (p. 1643) occurred only with the formation of a six-membered ring. No case was found in which a hydrogen bond compound was in equilibrium with an OH tautomer.

The study just discussed affords an excellent example of the utility of a physical measurement which yields definite information about the detailed structure of compounds. Since certain structural units are frequently responsible for definite isolated absorption bands, the use of absorption measurements is often much more conclusive for purposes of determining molecular configuration than the employment of a method such as the parachor. This is true because the parachor value represents the sum of all of the structural units in the molecule and hence necessitates that individual units be identified by taking differences, a procedure frequently lacking in accuracy.

Visible and Ultra-violet Absorption Spectra. As previously stated, the absorption of visible light is usually associated with a change in the electronic structure of one or several parts of the absorbing molecule, accompanied by alteration in the vibrations of the atoms in the groups and by variation in the molecular rotation. If only electronic excitation occurred the absorption would be confined to a single definite wave length; it is the additional changes in vibrations and rotation which are responsible for the broad nature of observed absorption bands. It frequently happens that there are two groups in the molecule which are more or less isolated and which act to a considerable extent as individual units. If the two groups are dissimilar and are sufficiently isolated in the sense that there is no electronic interaction between them, the absorption spectrum usually exhibits two separate absorption bands. If there is interaction between the groups the resultant absorption is characteristic of neither and shows a single modified band.

The influence of one group upon another as shown by absorption measurements is illustrated by observations on the absorption spectra of solutions of aldehydes.⁹⁹ The spectra of benzene, propionaldehyde, benzaldehyde, phenylacetaldehyde, and hydrocinnamaldehyde were investigated. It was found that the introduction of the aldehyde group into benzene profoundly modified the absorption of benzene. The separation of the CHO group from the benzene ring by a CH₂ group as found in phenylacetaldehyde resulted in less modification of the benzene absorption, and the introduction of two CH₂ groups resulted in an absorption spectrum in which the absorption due to the benzene ring was practically identical with that of benzene. Simultaneous with the above changes in the absorption of the benzene ring there occurred

⁹⁹ Arnold and Kistiakowsky, *ibid.*, **54**, 1713 (1932).

alterations in the absorption of the CHO group as found in propionaldehyde; in hydrocinnamaldehyde the carbonyl absorption was identical with that in propionaldehyde. The absorption spectrum of hydrocinnamaldehyde was the same as that of an equimolal mixture of benzene and propionaldehyde. It appears from these data that interaction effects are completely eliminated between the carbonyl and phenyl groups when they are separated by two methylenes.

Similar results have been obtained¹⁰⁰ in a study of the influence on absorption of the position of two groups in disazo dyes. It was found

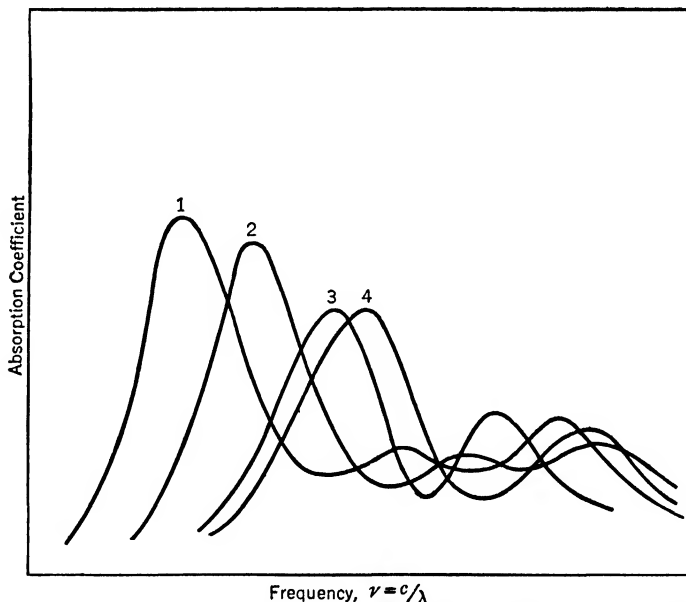


FIG. 13.*—Influence of separation of absorbing groups upon absorption spectrum.

- 1 = $\text{RN}=\text{NC}_6\text{H}_4\text{CH}=\text{CHC}_6\text{H}_4\text{N}=\text{NR}'$; 2 = $\text{RN}=\text{NC}_6\text{H}_4\text{C}_6\text{H}_4\text{N}=\text{NR}'$;
 3 = $\text{RN}=\text{NC}_6\text{H}_4\text{CH}_2\text{C}_6\text{H}_4\text{N}=\text{NR}'$; 4 = $\text{RN}=\text{NC}_6\text{H}_4\text{CH}_2\text{CH}_2\text{C}_6\text{H}_4\text{N}=\text{NR}'$
 $\text{R} = (\text{CH}_3)(\text{OH})\text{C}_6\text{H}_3-$; $\text{R}' = (\text{CH}_3)_2\text{NC}_6\text{H}_4-$.

that if the two groups are conjugated with each other the effect is the apparent formation of a new electronic system. If the groups are separated by one or two CH_2 units the effect is essentially that of addition of the separate absorbing systems. These results are illustrated in Fig. 13.

Almost all absorption data on organic compounds have been secured from the liquid form or from solutions. Under these conditions the detailed structure of an absorption band is lost because of the perturbing effects of surrounding molecules. These disturbing factors can be

¹⁰⁰ Piper and Brode, *ibid.*, **57**, 135 (1935).

* From Piper and Brode, *loc. cit.* (Courtesy of publishers.)

considerably removed by making measurements at very low temperatures in dilute solutions of non-polar solvents.⁹⁹ At low temperatures the thermal effects which are responsible for the disturbance of the vibrations of the atomic groups are small.

The study of the absorption spectra of organic compounds in the vapor state has been confined to relatively few substances, and the detailed analyses of the data have been restricted to very simple molecules. Although these have theoretical significance, their practical importance to the organic chemist is not great at present.

Whereas Raman shift data give information as to the types of bonds and atomic groups present in molecules, visible and ultra-violet absorption measurements are useful in identifying their integrated structures. The absorption spectrum of a compound is the resultant of all the various components which make up the molecule and is therefore a physical property which is characteristic of the entire molecule. The additive and constitutive parts of this physical property have not been analytically separated as yet.

The groups of atoms which act as units in determining the absorption characteristics of organic compounds are called chromophores. Examples of the better-known chromophores are $C=C$, $N=N$, $C=O$, $N=O$, and combinations of these linkages, e.g., $C=C-C=C$, $N \begin{smallmatrix} \nearrow O \\ = \end{smallmatrix}$, $C=C-N=N-C=C$, etc. In simple molecules it has been possible to assign rough absorption regions to the various chromophoric groups; for example, in aliphatic ketones and aldehydes the carbonyl group exhibits absorption in the general region 2600–3000 Å. As previously discussed, the exact position of the band due to a chromophore is influenced by neighboring and adjoining groups. Certain linkages such as $C=C$ and $C-H$ do not appear in visible absorption spectra although they are found in infra-red spectra. This is due to the superposition of the electronic shift upon the vibrational excitation; the energy necessary to cause a change in the electronic structure of the $C=C$ or $C-H$ linkage is relatively great, and the absorption band is far in the ultra-violet.

The effect of interaction of one type of chromophore with others of its kind is shown in a study of the absorption spectra of a series of compounds containing a furyl group separated from a carboxyl group by conjugated chains of increasing length.¹⁰¹ The absorption curves are shown in Fig. 14.

In Fig. 14 it is seen that increasing the length of the conjugated chain by the addition of successive $CH=CH$ groups causes a progressive

¹⁰¹ Hausser, *Z. tech. Physik.*, **15**, 10 (1934).

shift of the absorption maximum to the red end of the spectrum. This observation is consistent with the chemical behavior of conjugated systems (p. 575). As the number of conjugated linkages is increased, it becomes relatively more easy to produce electronic changes in the system; this means that the absorption will shift to the low-energy end of the spectrum, the red. Chemical evidence has shown that with increasing length the thermal reactivity of a conjugated system becomes progressively greater.

Conrad-Billroth has published¹⁰² a series of papers on the absorption spectra of aromatic compounds and has pointed out certain relation-

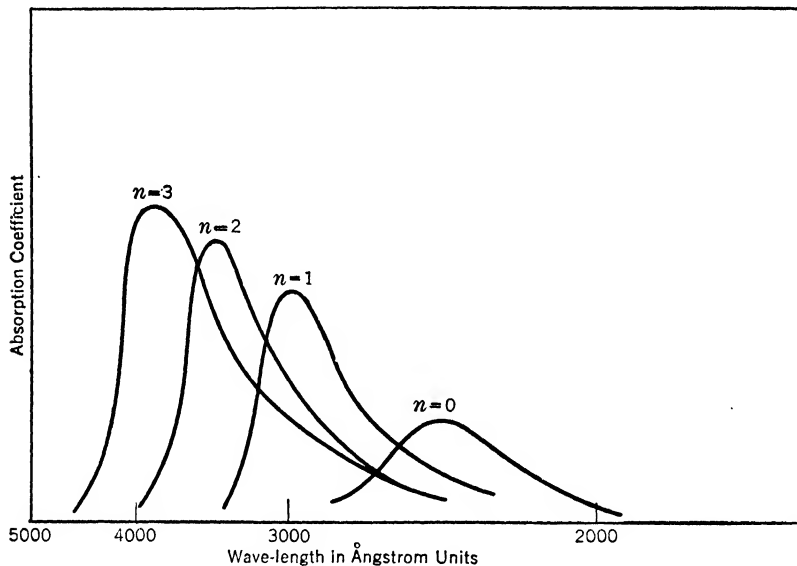
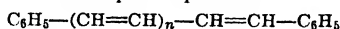


FIG. 14.*—Influence of increasing length of conjugated chain upon absorption spectrum.



ships which exist between the character of the absorption band of a compound and other physical properties such as the dipole moment. Attempts to resolve the absorption spectra of organic compounds into additive and constitutive components have not proved very successful.¹⁰³

The introduction of groups other than chromophores into a molecule which contains one or more chromophores can produce one of several effects. When the entering group merely increases the weight of the

¹⁰² Conrad-Billroth, *Z. physik. Chem.*, **B25**, 139 (1934); **B20**, 222 (1933); Hillmer and Schorning, *ibid.*, **A167**, 407 (1934).

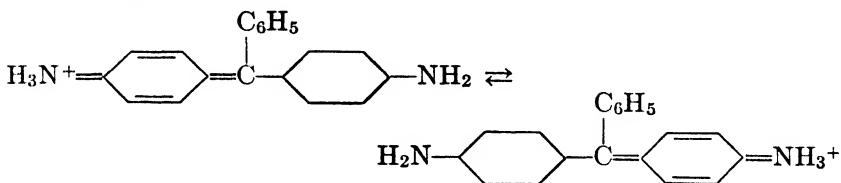
* Hausser, *loc. cit.* (Courtesy of publishers.)

¹⁰³ Wolf and co-workers, *Z. physik. Chem.*, **B13**, 201 (1931); **B21**, 389 (1933).

molecule there is generally a slight shift of the absorption bands to longer wave lengths or lower frequencies. The introduction of certain groups such as OH or NH₂ into a molecule containing chromophores frequently results in a marked change of the position and character of the absorption. This phenomenon has been discussed by Bury.¹⁰⁴

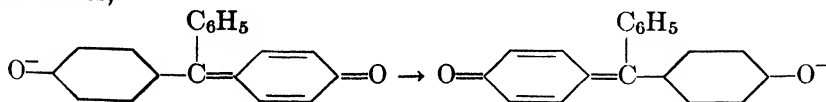
Bury has applied the concept of quantum mechanical resonance (p. 1857) as developed by Pauling and others to the consideration of the absorption spectra of certain dyes. When a molecule can have two structures which are of equal energy content and which can be derived one from the other without marked relocation of the atoms, the molecule is said to resonate between the two structures. The best-known example of resonance is the Kekulé structure of benzene (p. 55) in which the atoms remain stationary but the bonds oscillate between two positions. Bury has suggested that the presence of structural resonance in a molecule is responsible for the shifting of the absorption bands farther to the red than would be normally expected from a consideration of the chromophores present.

Döbner's violet, which is the hydrochloride of *p,p'*-diaminotriphenylcarbinol, can resonate between two equivalent structures.



This compound therefore has much deeper color than would be expected from a molecule containing the non-chromophoric NH₂ group and the three phenyl groups which absorb in the ultra-violet. The compound *p*-aminotriphenylmethane hydrochloride is colorless, and examination will show that no resonating structures of equal energy are possible. The introduction of the non-chromophoric NH₂ group to one of the unsubstituted phenyls immediately introduces the possibility of resonance, and the deep violet color of the dye results (p. 1888).

The color of the hydroxytriphenylmethane dyes is likewise due to resonance. For example, the anion of benzaurin can have the two structures,



and the color of the dye is much deeper than that expected from the

¹⁰⁴ Bury, *J. Am. Chem. Soc.*, **57**, 2115 (1935).

chromophores present. The introduction of the non-chromophoric OH group into the colorless sodium *p*-hydroxytriphenylmethane produces the deep color of the dye because of the formation of resonating structures. The introduction of OCH₃ into sodium *p*-hydroxytriphenylmethane does not produce color, and an examination of the structure of the product shows that no resonance is possible.

The concept of resonance as applied to the absorption spectra of dyes is extremely valuable. It furnishes an explanation of the so-called auxochromic action of such groups as the NH₂ and OH radicals and provides a theoretical basis for considerations of the relationships of color and chemical constitution.

Isomeric compounds of different energy contents usually exhibit different absorption characteristics. Thus, the absorption of a *trans* compound extends farther to the red than that of its *cis* isomer,¹⁰⁵ and the absorptions of keto and enol isomers are similarly unlike.¹⁰⁶ On the other hand, isomers of equal energy content, e.g., optical enantiomorphs, show identical absorption spectra.¹⁰⁷

The absorption spectrum of a compound in a non-polar solvent is usually independent of concentration effects as regards the intensity of absorption and the position of the bands. However, salt-like compounds may show variations in their absorptions with changes in the dielectric constant of the solvent due to differences in degrees of dissociation. There may also be variations in the absorption characteristics of a dissolved substance in a series of solvents due to differences in their electronic distribution; there is evidently interaction between the dissolved molecule and the electric fields set up by the solvent.¹⁰⁸ Some of these effects are illustrated in Fig. 15.

The usefulness of absorption spectrum data at the present time lies largely in the qualitative identification and quantitative estimation of organic compounds. For purposes of quantitative estimation the method is usually reliable and can be checked against standard preparations. For purposes of qualitative identification caution must be exercised in the use of absorption data, and great care must be employed in eliminating traces of impurities which are likely to cause confusion. Entirely too much optimism has been felt by many research workers as to the generality of the utility of absorption data; however, with the proper appreciation of the limitations of the method much valuable information can be derived from its use.

¹⁰⁵ Smakula, *Z. physik. Chem.*, **B25**, 90 (1934).

¹⁰⁶ Lowry and co-workers, *J. Chem. Soc.*, 1333 (1928); Morton and co-workers, *ibid.*, **127**, 2698 (1925); 706 (1926).

¹⁰⁷ Brode and Adams, *J. Am. Chem. Soc.*, **46**, 2032 (1924).

¹⁰⁸ Brode, *J. Phys. Chem.*, **30**, 56 (1926).

In the field of natural products the progress of many investigations has been due largely to the availability of the physical method of identification which absorption measurements supply (pp. 1180, 1358). The use of absorption data has proved valuable in the identification of isomers, for example, the pyrrole pigments.¹⁰⁹ As a method of determining the purity of a product the measurement of the absorption spectrum serves as a valuable tool since traces of impurity often give rise to bands which are not masked by the absorption of the principal substance.

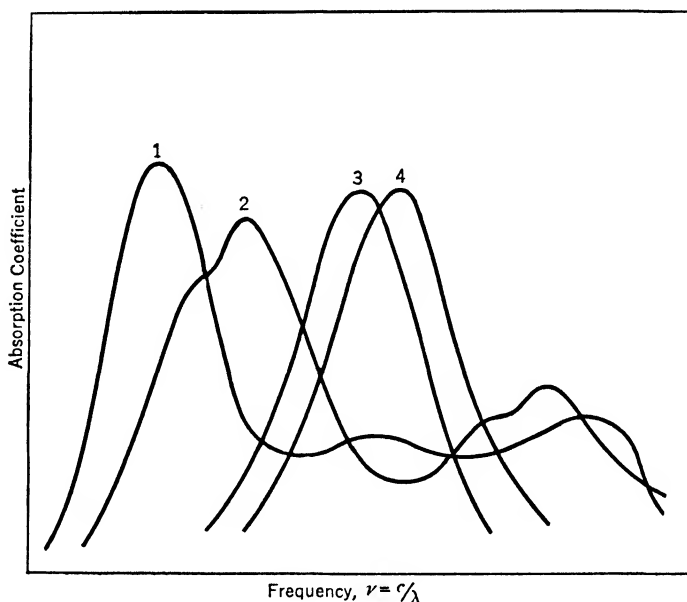


FIG. 15.*—Influence of solvent on the absorption spectrum of benzeneazophenol.

1 = HCl solution; 2 = pyridine solution; 3 = alcohol solution; 4 = KOH solution.

With the continued development of the theoretical principles underlying absorption effects much wider use of absorption data can be anticipated. The possibility of bringing out the fine structure of bands by making measurements at very low temperatures offers hope of widening the scope of utility of the absorption method. Finally, the proper combination of data derived from Raman effect and from infra-red, visible, and ultra-violet absorption measurements provides a method of analysis which may enable the resolution of total effects into their additive and constitutive parts.

¹⁰⁹ Stern and Wenderlein, *Z. physik. Chem.*, **A170**, 337 (1934); **A174**, 81 (1935); **A177**, 40, 165 (1936).

* From data of Brode, *loc. cit.* (Courtesy of publishers.)

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CHAPTER 21

ROTATORY DISPERSION

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PART I. THEORIES OF OPTICAL ACTIVITY

The requirements for the production of optical rotation in organic molecules are given in the general chapter on stereochemistry (p. 155). In Part I of the present chapter the physical theory of optical activity is discussed as well as its application in dissecting the optical rotation of the molecule as a whole into the rotatory contributions of each individ-

ual chromophoric group. Part II deals with the analysis of the partial rotation of the significant group of organic molecules. This information should serve, on one hand, for testing the validity of the present or future physical theories of optical activity; on the other, for the correlation of the configurations of chemically related substances.

The theories of optical activity deal with two distinct phases of the phenomenon: the physical nature of the optical rotation and the conditions necessary to produce it.

Nature of Optical Rotation

Kinetic Interpretation of Fresnel. A few years after the discovery of optical rotation by Arago and Biot, the phenomenon was kinetically interpreted by Fresnel as "circular double refraction." Fresnel¹ showed that a beam of plane polarized light can be looked upon as the sum of two circular vibrations of opposite sense and equal amplitude, the phase being such that the circular vectors meet on the direction of polarization *OR*. (See Fig. 1A.) Algebraically, a right circularly polarized ray may be represented by the following equations:

$$E_x^r = E_0 \cos 2\pi\nu t, \quad E_y^r = -E_0 \sin 2\pi\nu t$$

where E_x^r and E_y^r are the *x* and *y* components of the rotatory vector E^r , ν being the frequency. Similarly, the equations for a left circularly polarized ray are as follows:

$$E_x^l = E_0 \cos 2\pi\nu t, \quad E_y^l = E_0 \sin 2\pi\nu t$$

The addition of the E_x and E_y components of the two rays, respectively, gives the equation of a plane polarized ray.

$$E_x = E_x^r + E_x^l = 2E_0 \cos 2\pi\nu t, \quad E_y = E_y^r + E_y^l = 0$$

In a rotatory active medium, the velocities of the two circularly polarized rays are different, and so the plane of polarization of the emergent plane polarized light is not the same as that of the incident beam. This is illustrated in Fig. 1B, in which the direction of the light is perpendicular to the plane of the paper, the light going towards the observer.

In Fig. 1B, the left circular vibration is retarded with respect to the right, the vector OM_2 being retarded by an angle 2α . The resulting vector OR has been turned to the right through an angle α . The angle of rotation of the resulting vibration is given by the following expression:

$$\alpha = \frac{\omega}{2} \left(\frac{l}{v_l} - \frac{l}{v_r} \right) \quad (1)$$

¹ Fresnel, *Ann. chim. phys.*, [2], **28**, 147 (1825).

where l is the length of the medium, v_r and v_l are the velocities of *dextro* and *levo* circularly polarized rays, and ω is the angular velocity of the rotating vector. Substituting $2\pi\nu$ for ω , λ for $\frac{c}{\nu}$, $\frac{c}{n_l}$ for v_l , and $\frac{c}{n_r}$ for v_r , (n_l and n_r being the refractive indices for the two circularly polarized rays and c the velocity of light in vacuo), equation (1) becomes:

$$\alpha = \frac{\pi l}{\lambda} (n_l - n_r) \quad (2)$$

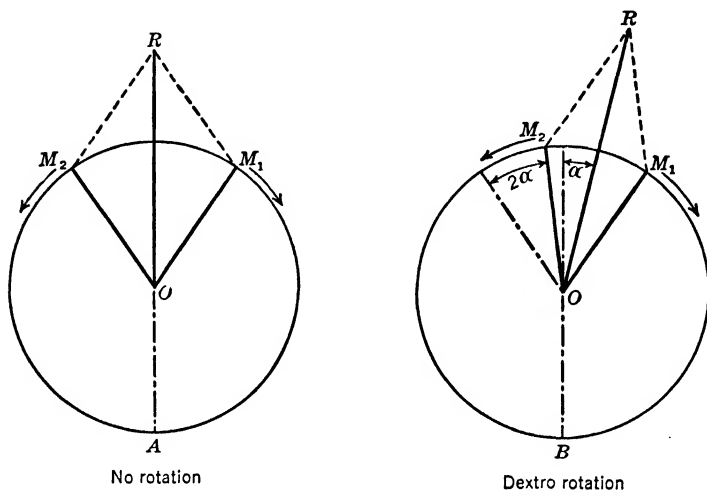


FIG. 1

(where α is expressed in radians). The difference $n_l - n_r$ is very small as compared with the value of α . A quartz plate 1 mm. thick, for instance, has an optical rotation of 21.7° for λ 5892.6, hence,

$$n_l - n_r = \frac{21.7 \times 5893}{180 \times 10^7} = 0.000071$$

It follows that optical activity is a very small disturbance of ordinary refraction. The events are more complicated in a region of absorption, but it will be shown presently that there, analogously, circular dichroism is a small disturbance of ordinary absorption.

Circular Dichroism. For a light of wave length falling within a region of absorption of an optically active molecule, not only the velocities of the two circularly polarized rays but also, in certain instances, their respective absorption coefficients are different. In the latter case, the emergent ray is not plane but elliptically polarized, and the phenome-

non is generally known as *circular dichroism*. In Fig. 2 the left circularly polarized ray has been assumed to be more strongly absorbed. A simple calculation shows that to a first approximation the ellipticity is given by the following expression:

$$\phi = \frac{\pi l}{\lambda} (\kappa_l - \kappa_r) \quad (3)$$

where κ_l and κ_r are the absorption indices (as defined in the International Critical Tables) of the two circularly polarized rays. This formula is analogous to the one derived for the expression of the rotatory power in a region of transparency.

Origin of Optical Activity

A complete theory of optical activity should predict the whole rotatory dispersion curve of an active molecule (curve of rotation as a function of wave length). It should also predict the absolute configurations of optically active molecules from their signs of rotation, this being one of the ultimate aims of stereochemistry. As recently as 1933, Freudenberg, in his "Stereochemie," wrote, "The knowledge of the absolute configuration of molecules is beyond chemistry," and yet definite progress in that direction has already been made.

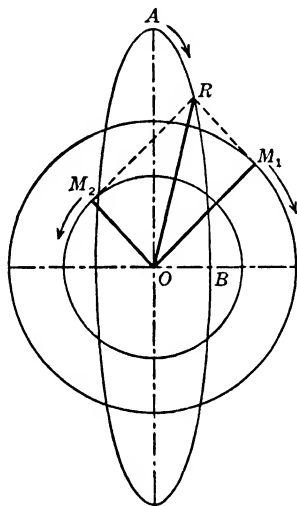


FIG. 2

OLDER THEORIES

Theories of Guye and Brown. Following up the ideas of Fresnel, Pasteur formulated his general principle of dissymmetry; later van't Hoff introduced his concept of the asymmetric carbon atom as a necessary condition for optical activity. The theories of Crum Brown² and of Guye³ were an early attempt to obtain a quantitative relation between the optical activity of a molecule with one asymmetric carbon atom, and some parameters of the asymmetry of the molecule. Guye postulated that the masses of the four radicals attached to the central carbon atom were a measure of this asymmetry, and his formula states

² Brown, *Proc. Roy. Soc. Edinburgh*, **17**, 181 (1890).

³ Guye, *Compt. rend.*, **110**, 714 (1890).

that the rotation should be proportional to a "product of asymmetry" of the form:

$$P = \frac{(m_1 - m_2)(m_1 - m_3)(m_1 - m_4)(m_2 - m_3)(m_2 - m_4)(m_3 - m_4)}{(m_1 + m_2 + m_3 + m_4)^6}$$

where the numerals 1, 2, 3, and 4 indicate the position on the central carbon atom. It is evident that, when any two substituents are equal, the rotation becomes zero, and furthermore, if any one of the involved six differences changes sign, the sign of the rotation is also changed.

The theories of Crum Brown and of Guye were soon abandoned when it was discovered that isomeric radicals of equal mass are not equivalent in their effect on optical rotation. It may be noted, however, that in a restricted group of substances Guye's rule does hold.

An instance is the series of hydrocarbons of the type
$$\begin{array}{c} \text{R}_1 \\ | \\ \text{H}-\text{C}-\text{R}_2 \\ | \\ \text{R}_3 \end{array}$$

(where R_1 , R_2 , and R_3 are normal aliphatic radicals) since in this case the changes in mass of the radicals go parallel with changes in their electronic structure.

All the theories which have superseded those of Brown and of Guye have a common origin: the electromagnetic theory of light. They all lead to analogous rotatory dispersion formulas in a region of transparency.

Biot's Law—Course of the Rotatory Dispersion Curves. From experiments on the rotatory dispersion of quartz, Biot recognized that the rotation was inversely proportional to the square of the wave length. This rule is known as Biot's law and may be expressed by $\alpha = A/\lambda^2$. Had the measurements of Biot been more accurate, there is no doubt that he would have found empirically the simplified Drude formula, $\alpha = \frac{A}{\lambda^2 - \lambda_0^2}$, which is now used to express accurately the rotatory dispersion of a great many compounds.*

Cotton Effect—Natanson's Rule. Cotton⁴ was the first to study experimentally the course of the rotatory dispersion in a region of absorption. The compounds he investigated were complex salts of tartaric acid. He observed that in potassium chromium tartrate

* It must be added, however, that Biot found that the rotatory dispersion of tartaric acid could not be expressed by his formula.

⁴ Cotton, *Ann. chim. phys.*, **8**, 347 (1896).

for decreasing wave lengths the rotation increased up to a maximum near the absorption band, became zero, and then increased in the other sense to a second maximum, as represented in Fig. 3.

He correlated this anomaly in the rotatory dispersion curve with the anomalous circular refraction and the circular dichroism observed in the same wave length interval. These phenomena are known under the name of *Cotton Effect*. Cotton has shown that the shape of the rotatory dispersion curve in the absorption band is easily deduced from the dispersion of the indices of refraction of the two circularly polarized rays. (See equation 2.)

In Fig. 4 are plotted the curves,

$$n_l = f_l(\lambda), \quad n_r = f_r(\lambda)$$

and the resultant curve,

$$\alpha = f(n_l - n_r)$$

Cotton also measured the circular dichroism in the region of absorption of the same and of other tartrates and found quite generally that the ellipticity was maximum at that wave length which corresponds to the point of inversion of the rotatory dispersion curve. Later on, Natanson showed that the sign of the circular dichroism determined the direction of the anomaly of the rotation:

On the long-wave-length side of the absorption band, the rotation is

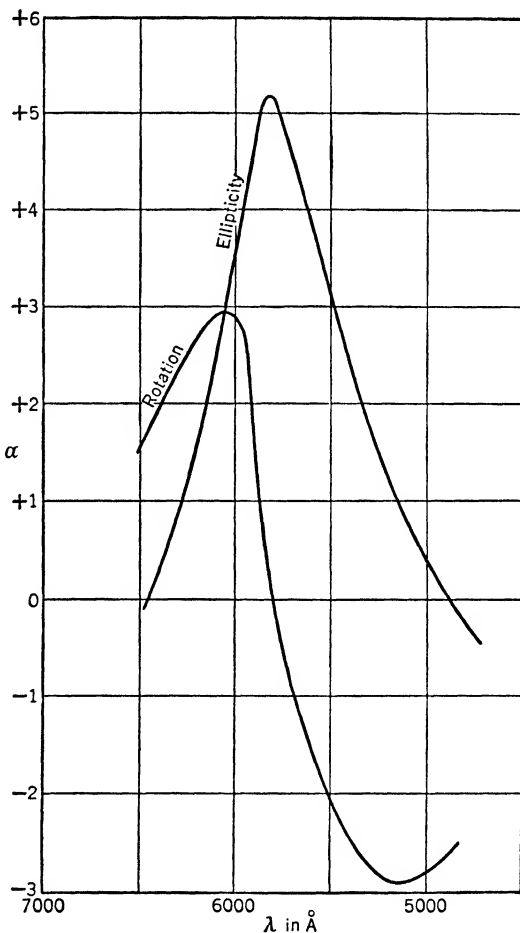


FIG. 3*

* Cotton, *Ann. chim. phys.*, **8**, 347 (1896). (Courtesy of publishers.)

dextro if the left circularly polarized beam is the one more strongly absorbed, and *vice versa*. This statement is known as Natanson's rule.

It must be borne in mind that the optical rotatory power of active molecules is the sum of partial contributions, each of which originates in a definite absorption band. Therefore, it very often happens that the "Cotton effect" in a band is partially masked by other rotatory

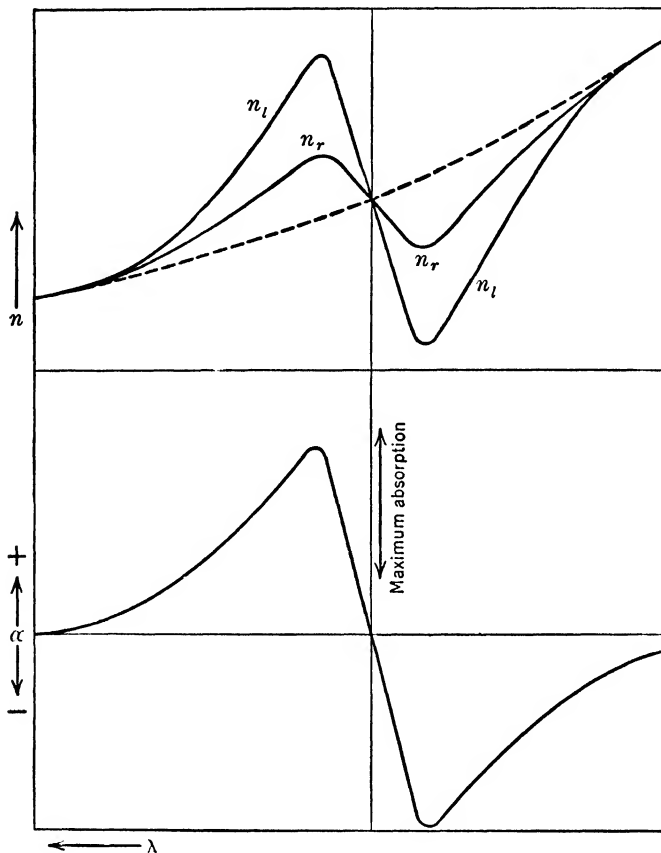


FIG. 4

contributions, and that the observed rotatory dispersion within the band does not represent the true character of the anomaly. On the other hand, accurate information concerning the rotatory contribution of a band can be obtained through a study of its circular dichroism since, as will be seen, formulas have been developed correlating dichroism and rotation quantitatively. It may also happen that an absorption band has no rotatory contribution. In such bands "Cotton effect"

(anomaly of the rotatory dispersion curve and presence of circular dichroism) can not be detected.

Drude's Formulas. Drude was the first to derive rotatory dispersion formulas by modifying in an appropriate way the dispersion formulas of ordinary refraction obtained by Maxwell, Sellmeier, Ketteler, and Helmholtz. The presence in the active molecule of oscillators (electrons) with a definite period of vibration was his fundamental assumption. He also postulated that the active electron, in order to respond differently to a *dextro* and a *levo* circularly polarized ray, had to vibrate along a helicoid path. Although his mathematical treatment does not correspond to his model, as has been recently pointed out by Kuhn⁵ (in fact, his model is optically inactive), the formulas he derived are valid. In a more convenient form given by Natanson⁶ and Bruhat,⁷ they are as follows:

$$\alpha = \frac{\pi\nu_0^2}{c^2} \nu^2 \frac{\nu_0^2 - \nu^2}{(\nu_0^2 - \nu^2)^2 + \nu^2\nu'^2} D, \quad \phi = \frac{\pi\nu_0^2}{c^2} \nu^2 \frac{\nu\nu'}{(\nu_0^2 - \nu^2)^2 + \nu^2\nu'^2} D \quad (4)$$

where α is the angle of rotation, ϕ is the ellipticity in a region of absorption, D is a constant, ν_0 is the head of the absorption band, and ν' is the damping factor. More than one band should usually be taken into account to express the rotation of the simplest molecule. Thus a sum of terms of the above type, each term with its own D , ν' , and ν_0 values, has to be considered.

Outside an absorption band, the term $\nu\nu'$ may be neglected, the ellipticity is zero, and the expression for the rotatory dispersion becomes

$$\alpha = \frac{\pi\nu_0^2}{c^2} \frac{\nu^2}{\nu_0^2 - \nu^2} D \quad (5)$$

or in terms of λ , $\alpha = \frac{A}{\lambda^2 - \lambda_0^2}$, where $A = \pi D$. This expression is known as the *simple Drude formula*; the total rotation of the molecule is a sum of partial terms of the above type, such as,

$$[M] = \sum \frac{A}{\lambda^2 - \lambda_0^2} \quad (6)$$

This "simple Drude formula," or better, a sum of two or three such terms, was shown by Lowry and others to express with accuracy the rotatory dispersion of many organic compounds. Inside the band, $\nu_0^2 - \nu^2$ is small, and the term $\nu\nu'$ cannot be neglected. The analysis

⁵ Kuhn, *Z. physik. Chem.*, **B 20**, 325 (1933).

⁶ Natanson, *J. phys.*, [4], **8**, 321 (1909).

⁷ Bruhat, *Ann. phys.*, **3**, 232 (1915).

of the formula leads to the following conclusions, as pointed out by Bruhat:⁷

1. The rotation becomes zero and changes sign for $\nu = \nu_0$.
2. The rotation exhibits one maximum and one minimum for values of ν approximately equal to $\nu_0 \pm \nu'/2$ where the ellipticity has half its maximum value.
3. The difference between the maximum and the minimum rotations is approximately equal to the maximum ellipticity.

It can be seen in the few instances where the observed rotation is furnished mainly by one absorption band that the shape of the experimental rotatory dispersion and of the ellipticity curves inside the band is qualitatively of the type required by the above formulas. One cannot expect a perfect agreement between these formulas and experimental values for the following reasons:

First: These formulas are valid for the gaseous state and not for the liquid phase.

Second: It is well known that formulas of the Ketteler-Helmholtz type do not accurately represent experimental absorption curves (p. 1765). Hence it cannot be expected that the Drude formulas which are analogous to the Ketteler-Helmholtz dispersion formulas should express with precision the rotatory dispersion curve inside an absorption band. In potassium chromium tartrate, the rotatory power in the green yellow part of the spectrum is mainly furnished by one partial contribution, as may be seen by the symmetry of the dispersion curve. (See Fig. 3.) If the Drude formulas held accurately, then one should expect the value of the rotation at the maximum to be about half the value of the maximum ellipticity. As may be seen, the rotation reaches 3° , and the maximum ellipticity is 5.3° . The discrepancy is probably still greater according to newer measurements made by Kuhn and Szabo.⁸ As will be seen later, the newer rotatory dispersion formulas developed by Kuhn are in better agreement with the experimental data.

NEWER THEORIES

Although the theories that came after that of Drude have a greater significance for the physical theory of optical rotation than for their application to the problems of organic chemistry, yet they should be discussed here since they were the basis of the theory of Kuhn which had such a profound influence on organic research.

⁸ Kuhn and Szabo, *Z. physik. Chem.*, **B 15**, 59 (1931).

Theories of Oseen and of Born. Among the newer theories, first place should be given to those of Oseen⁹ and of Born.¹⁰ The following conditions are essential in both theories: First, the dimensions of the molecules cannot be neglected when compared to the wave-length of the incident light as in the ordinary theories of dispersion. Second, the different resonators located in a molecule are coupled; that is, the displacement of one resonator influences the displacement of the other. At least four "non-planar" electrons are necessary to produce optical activity, a condition which is fulfilled by the asymmetric carbon atom.

J. J. Thomson's Theory.¹¹ This theory also assumes a coupling between resonators, and furthermore postulates in the optically active molecule two dissymmetric systems, one produced by a rigid tetrahedron of the four groups attached to the asymmetric carbon atom, the other corresponding to the valence electrons of the four bonds.

de Malleman's Theory.¹² The original assumptions of de Malleman's theory are similar to those of Born, i.e., the existence of coupling forces between the different resonators and the influence of the dimension of the molecule. His model is an irregular tetrahedron whose edges are determined by the dimensions of the substituent radicals or substituent atoms. de Malleman attributes an electric vector to each of the four radicals or atoms. The electric moment of an atom is the sum of the moments of its valence electrons. This moment corresponds to the refractivity of the atom. de Malleman calculated the molecular rotation of the hypothetical compound CHClBrI and found $[\alpha]_D \simeq 8^\circ$. This value seems to be of the right order of magnitude, but it cannot be verified experimentally since the compound is unknown. It is interesting to note that rotatory power in this theory depends to a first approximation on the inverse eighth power of the distance between the atoms, a relation obtained by Born in his latest calculations.

The weakest part in de Malleman's theory is his assumption concerning the polarizabilities of the atoms. He had to introduce this assumption to calculate the rotation from known physical properties. The weak absorption bands situated in the near ultra-violet region, which have an insignificant effect on the refractivity of a radical, may lend a very important contribution to the rotatory power. The effect of such bands is entirely neglected in this theory.

Boys' Theory.¹³ Like de Malleman, Boys endeavors to find a

⁹ Oseen, *Ann. Physik*, **43**, 1 (1915).

¹⁰ Born, *Physik. Z.*, **16**, 251 (1915).

¹¹ Thomson, *Phil. Mag.*, [6], **40**, 713 (1920).

¹² de Malleman, *Compt. rend.*, **181**, 298 (1925).

¹³ Boys, *Proc. Roy. Soc. (London)*, **A 144**, 655 (1934).

relation which will express the total rotation of an active molecule as a function of the refractivities of the groups and of their spatial distribution. His model consists of a tetrahedron of four isotropic particles (atoms or radicals). All the tetrahedra are distributed at random so that the medium as a whole is isotropic.

Under the action of the electric field of a light wave, each atom becomes an oscillating electric doublet. Inside the molecule, the electric field of the wave is altered by the fields of the doublets themselves. In other words, the secondary wavelets caused by all the doublets, when added together, change the plane of polarization of the original wave, if the model is asymmetric.

The calculation of Boys led to the following formula for the specific rotation of a medium consisting of molecules containing one asymmetric carbon atom.

$$[\alpha] = \frac{16.62 (n^2 + 2)(n^2 + 5) R_A R_B R_C R_D (1 + F)}{\lambda^2 M (a + b + c + d)^{14}} \quad (7)$$

where M is the molecular weight of the substance; n is the refractive index of the medium; R_A, R_B, R_C, R_D are the refractivities of the groups A, B, C, D (as given by the formula of Lorenz and Lorentz [p. 1738]); a, b, c, d are the effective radii of the same groups in Ångström units; and F is a function of the distances a, b, c, d which has not much effect. Its values may be found in Boys' original article.

It is apparent from this formula that only the refractivities and the diameters of the four radicals are necessary to calculate the rotation. The sequence A, B, C, D determines the spatial distribution of the groups. By convention, the order B, C, D appears clockwise when A is towards the observer. Consequently, the rotation is *dextro* when $A > B > C > D$, since all the six differences $(a - b)(a - c)$, etc., have a positive value. Thus the formula allows the determination of the absolute configuration of a molecule from the sign of rotation of the latter. The rule may be expressed as follows: *A compound is dextro-rotatory when, viewed with the largest group towards the observer, the arrangement of the three other groups appears clockwise in the order of diminishing volume.*

The assumption that the four radicals can be looked upon as isotropic spheres seriously limits the application of Boys' equation (7). Furthermore, it appears from this equation that the dispersion of the rotatory power should be given by the dispersion of the product,

$$\frac{R_A R_B R_C R_D}{\lambda^2}$$

because this theory, like that of de Mallemann, neglects the influence of the absorption bands unimportant for the refractivity of a group. Born, discussing Boys' formula, remarks that rotatory power and refractivity are both "sums of terms of the type $\frac{a_i}{\nu_i^2 - \nu^2}$ ", whereas, in the case of the refractive index, the a_i are all positive, in the case of rotatory power their sum is zero so that some of them must be negative." The result is that the two dispersions cannot be expressed identically.

Kuhn's Theory. Kuhn,¹⁴ like the preceding authors, postulated a coupling between electrons located in different parts of the same molecule.

The progress made by the theoretical considerations of Kuhn is the development of new formulas to express the circular dichroism and the rotatory dispersion inside an absorption band. The method followed consists in finding the simplest oscillating system which would react differently towards a right-handed and a left-handed circularly polarized wave and thus be optically active. The model used consists of two harmonic oscillators of charge e_1 and e_2^* and mass m_1 and m_2 separated by a distance d along the z axis. The first resonator is then vibrating along the x axis and the second in the direction of the y axis. The total potential energy of the system is

$$U = U_1 + U_2 = \frac{1}{2}k_1x_1^2 + \frac{1}{2}k_2y_2^2$$

$$\nu_1 = \frac{1}{2\pi}\sqrt{\frac{k_1}{m_1}} \quad \nu_2 = \frac{1}{2\pi}\sqrt{\frac{k_2}{m_2}}$$

This particular system exhibits no optical activity. However, when the vibrations of both resonators influence each other, so that they are coupled, then a third term has to be added to the equation representing the potential energy of the system.

$$U = \frac{1}{2}k_1x_1^2 + \frac{1}{2}k_2y_2^2 + k_{12}x_1y_2$$

The forces acting on resonators one and two are now

$$k_1 = -k_{11}x_1 - k_{12}y_2 \quad \text{and} \quad k_2 = -k_{22}y_2 - k_{12}x_1$$

From the action of these two forces arise two vibrations, ξ_1 and ξ_2 , with approximate frequencies ν_1 and ν_2 if the coupling force is small.

¹⁴ Kuhn, *Trans. Faraday Soc.*, **26**, 293 (1930).

* The values $\frac{e_1}{m_1}$ and $\frac{e_2}{m_2}$ can be written $\frac{e_1}{m_1} = f_1 \frac{e}{m}$ and $\frac{e_2}{m_2} = f_2 \frac{e}{m}$, where f_1 and f_2 represent the number of optical electrons per molecule. An f value is a measure of the intensity of a band and may be called "intensity factor" of the oscillator.

The important feature is that both resonators take part in each vibration. Analysis shows that if $\nu_2 > \nu_1$, then the forced motion of resonator 2 (in vibration ξ_1) is along the negative axis of y and the forced motion of resonator 1 (in vibration ξ_2) is along the positive axis of x . (The opposite signs for the two forced motions result from the fact that the resonance terms $(\nu_2^2 - \nu_1^2)$ and $(\nu_1^2 - \nu_2^2)$ are of opposite sense.)

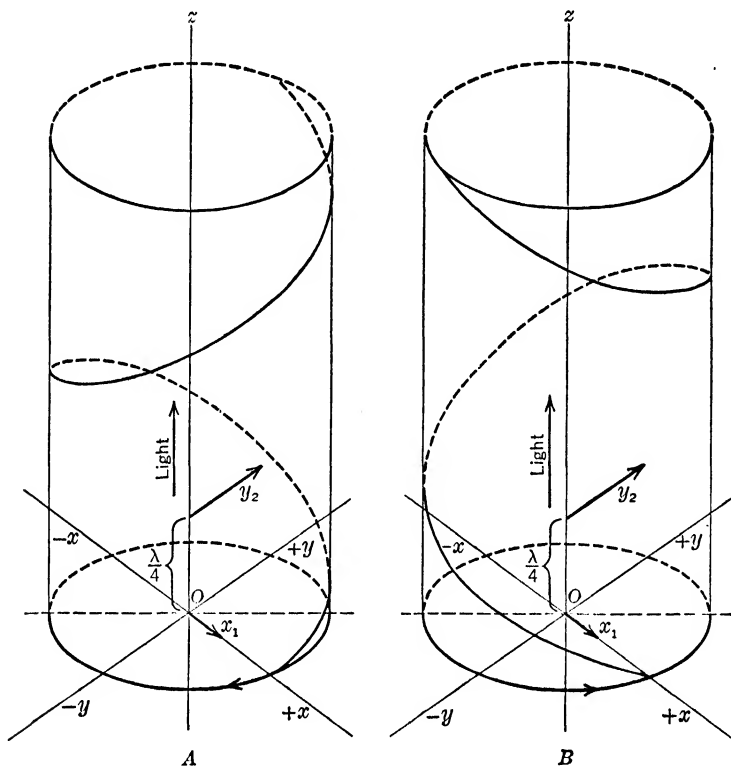


FIG. 5

It can be shown that, if a plane polarized wave is incident on the system, the work transferred to particles 1 and 2 in either vibration ξ_1 or ξ_2 is different for the right and left circularly polarized components of the wave. This result is graphically represented in Fig. 5. The action of a right wave acting on vibration ξ_2 may be seen in Fig. 5A; in Fig. 5B appears the action of a left wave acting on the same vibration.

The forced motion of particle 1 is represented by ox_1 . The sense of rotation of the two electrical vectors corresponding to the two circularly polarized rays—as a function of time, d being constant—has been indicated by arrows on the circles drawn in the plane XOY .

To render the phenomenon more apparent, the distance d has been chosen equal to $\lambda/4$.

It is evident that the two actions are not symmetric, since for $z = d$ the sign of the vector of the right circularly polarized wave acting on particle 2 is the same as the sign of the velocity of y_2 , whereas the vector of the left circularly polarized wave is of opposite sign. Hence there should be a difference in the indices of refraction of the two waves and the model should exhibit rotation.

It follows immediately that *a molecule is optically active if the vibrating moment corresponding to a given absorption band has at least two non-parallel components.*

The calculation takes account of the vibrations set up under the influence of light and of the secondary waves originating from the resonators. The method is closely similar to that followed in the theory of ordinary dispersion. There, too, a damping factor has to be added in order to obtain a general equation valid in regions of absorption.

Notwithstanding the difference between the models of Drude and of Kuhn, the latter arrives at expressions for optical rotation and circular dichroism identical with those of Drude if the various constants of his equations are included in the constant D of Drude's equations. Kuhn has shown that the constant could also be expressed as a linear function of a factor g defined as

$$(g)_{\xi_1} = (g_1)_{\xi_1} \frac{\nu}{\nu_1} = \left(\frac{n_l - n_r}{n - 1} \right)_{\xi_1} = \left(\frac{\mu_l - \mu_r}{\mu} \right)_{\xi_1} \quad (8)$$

where g_1 is the value of g for ν_1 , and $(\mu_l)_{\xi_1}$ and $(\mu_r)_{\xi_1}$ are the absorption coefficients of the two circular components of the vibration ξ_1 . These absorption coefficients are related to the ordinary absorption coefficient μ (as defined in the International Critical Tables) by $\frac{\mu_l + \mu_r}{2} = \mu$, and $(n_l)_{\xi_1}$ and $(n_r)_{\xi_1}$ are the indices of refraction of the same components.

Kuhn calls the factor g the "anisotropy factor." For the constant D he arrives at the expression $D = \frac{Ne_0^2 c}{2\pi m} f_{\xi_1} \frac{g_1}{\nu_1^3}$. For a single vibration, say ξ_1 , the expressions for rotation and circular dichroism then become

$$\begin{aligned} (\alpha)_{\xi_1} &= \frac{Ne_0^2}{2mc} \frac{f_{\xi_1} g_1}{\nu_1} \nu^2 \frac{\nu_1^2 - \nu^2}{(\nu_1^2 - \nu^2)^2 + \nu^2 \nu_1'^2}, \\ 4(\phi)_{\xi_1} &= (\mu_l - \mu_r)_{\xi_1} = \frac{2Ne_0^2}{mc} \frac{f_{\xi_1} g_1}{\nu_1} \nu^2 \frac{\nu \nu_1'}{(\nu_1^2 - \nu^2)^2 + \nu^2 \nu_1'^2} \end{aligned}$$

where the damping factor ν'_1 is equal to the half-width of the absorption band, and ϕ is the ellipticity. The other constants have already been defined.

The sum of two terms of the above type represents the rotatory contribution of the vibrations ξ_1 and ξ_2 . For a region of transparency their sum can be expressed as follows:

$$\alpha \simeq \frac{Ne_0^2}{2mc} \left[\frac{f_{\xi_1} g_1}{\nu_1} \frac{\nu^2}{\nu_1^2 - \nu^2} + \frac{f_{\xi_2} g_2}{\nu_2} \frac{\nu^2}{\nu_2^2 - \nu^2} \right]$$

For a sum of several absorption bands the expression becomes

$$\alpha \simeq \frac{Ne_0^2}{2mc} \sum \frac{f_{\xi_i} g_i}{\nu_i} \frac{\nu^2}{\nu_i^2 - \nu^2} \quad (9)$$

Comparing Kuhn's rotatory dispersion formulas with those of ordinary refraction and absorption, the anisotropy factor can be formulated in the following way:

The anisotropy factor is the coefficient by which the contribution of one absorption band to the ordinary refraction has to be multiplied, in order to obtain the rotatory contribution of the same band. Or the anisotropy factor is the coefficient by which the ordinary absorption in a band has to be multiplied in order to obtain the circular dichroism of this band for the corresponding wave length. If the molecule had a plane of symmetry, the anisotropy of each band would of course vanish.

Furthermore, it can be deduced that

$$\frac{f_{\xi_1} g_1}{\nu_1} = - \frac{f_{\xi_2} g_2}{\nu_2},$$

and, by generalizing,

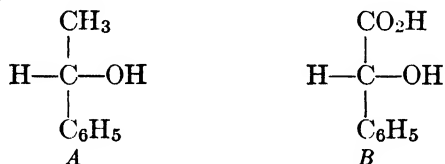
$$\sum \frac{f_{\xi_i} g_i}{\nu_i} = 0 \quad \text{or} \quad \sum A_i = 0 \quad (10)$$

The sum of the numerators of all the Drude terms corresponding to all the active bands of the molecule is zero. Or to each positive contribution $\frac{A\nu^2}{\nu_1^2 - \nu^2}$ furnished by vibration ξ_1 (absorption band ν_1) there corresponds a negative contribution $\frac{A\nu^2}{\nu_2^2 - \nu^2}$ originating in the coupled vibration ξ_2 (absorption band ν_2). These deductions offer an explanation for the low values of observed rotations.

It can easily be seen that the molecular rotation should vanish

towards both ends of the spectrum. For very low frequencies the numerators of each Drude term approach zero, while for high frequency the limit of each Drude term is A , and their sum is zero; hence $[M] = 0$.

The Vicinal Function. Tschugaeff has emphasized that, when a group with a definite absorption band is introduced into an active molecule, this band need not invariably acquire a partial rotation of its own. As has been seen, it becomes anisotropic if it is coupled. This anisotropy is termed by Kuhn "induced anisotropy." The vibration corresponding to this absorption band, however, by the mere fact that it is anisotropic, influences the "induced anisotropies" of the vibrations of the other absorption bands with which it is coupled. This effect is termed by Kuhn "vicinal function." Thus the coupled absorption bands of each substituent play a double role in the total molecular rotation. Methylphenylcarbinol (*A*) and mandelic acid (*B*) may serve as an illustration.



In *A* the absorption bands of the phenyl group at $\lambda \simeq 2600$ are not active. They become active, however, by the substitution of CO_2H for CH_3 . On the other hand, the carboxyl group acquires an anisotropy of its own different from that of the CH_3 group.

It is self-evident that individual substituents furnish different contributions to the total rotation, otherwise the molecular rotation would equal zero. According to Kuhn, the absorption bands in the Schumann region furnish the smallest contributions, and since the number of their dextrorotatory is nearly equal to that of their levorotatory contributions, the total contribution of the distant absorption bands in many instances may be negligible. In Kuhn's terminology they contribute principally by their vicinal effect. Kuhn further postulates that, inasmuch as the vicinal effect is produced principally by the bands in the Schumann region, it is not affected by small chemical changes in the substituents. However, "small change," according to Kuhn himself, is very difficult to define.

It must be added that the present-day theory of optical rotation does not permit the prediction of the vicinal effect of any given arrangement of substituents. Such information would involve complete knowledge of the relationship of chemical structure to optical activity. All the available information in this direction thus far has been obtained by

empirical methods, save the very few simple substances for which an absolute configuration has been derived.

The Rotatory Dispersion Curves of Liquids and Solutions. The equations obtained so far for the rotatory dispersion include the assumption that the absorption curves are of the Ketteler-Helmholtz type. Experience has shown that for substances in the liquid state this is not so. The following equation is the most general one representing the rotatory contribution of one absorption band in regions of transparency and of absorption, whatever the shape of the absorption curve.

$$\alpha = \frac{1}{2\pi} \int \frac{g_{\nu_1}}{\nu_1} \frac{(\nu_1^2 - \nu^2) \nu^2}{(\nu_1^2 - \nu^2)^2 + \nu^2 \nu_1'^2} \mu_{\nu_1} d\nu_1$$

This equation after simplification reduces to,

$$\alpha = \frac{1}{2\pi} \nu^2 \int_{\nu_1=\infty}^{\infty} \frac{g_{\nu_1}}{\nu_1} \frac{1}{\nu_1^2 - \nu^2} \mu_{\nu_1} d\nu_1$$

The integration has to be carried out over the frequency interval of the absorption band. Bielecki and Henri¹⁵ were the first to note that the formula of Ketteler-Helmholtz does not represent accurately the shape of the absorption curves of a number of organic compounds and that an exponential equation based on a Maxwellian distribution more satisfactorily expresses the experimental results.

The absorption curve can be represented by the following equation:

$$\mu = \mu_{\max.} e^{-\left(\frac{\nu - \nu_0}{\theta}\right)^2}$$

where $\theta = \frac{\nu'}{1.665}$. * (ν' = half-width of the band.)

The integration is carried out on the assumption that $g_{\nu_1} = g_0 \frac{\nu_1}{\nu_0}$ — in other words, that the anisotropy factor is practically constant within the band in spite of the fact that the same band could be resolved into its components (vibration and rotation terms) for the gaseous state. The resulting equation is

$$[M] = \frac{\Phi}{0.541} \frac{\nu}{\nu_\phi} \left[e^{-\left(\frac{\nu_0 - \nu}{\theta}\right)^2} \int_0^{\frac{\nu_0 - \nu}{\theta}} e^{x^2} dx - \frac{\theta}{2(\nu_0 + \nu)} \right] \quad (11)$$

where $[M]$ is the molecular partial rotation of the band ν_0 , and Φ the maximum value of this partial value which is reached for $\nu_\phi \simeq \nu_0 - 0.9\theta$.

¹⁵ Bielecki and Henri, *Physik. Z.*, **14**, 516 (1913)

* $\frac{1.665}{2}$ is the value of x which makes the term $e^{-x^2} = 0.5$.

At a certain distance from the absorption band (for practical purposes for $\nu < \nu_0 - 4\theta$) the integral is reduced to $\frac{\theta}{2(\nu_0 - \nu)}$ so that the equation takes the form of a simple Drude term,

$$[M] = \frac{\Phi}{0.541} \frac{\theta}{\nu_\phi} \left[\frac{\nu^2}{\nu_0^2 - \nu^2} \right]$$

It follows that the rotation outside an absorption band does not depend on the shape of the latter. The relation between maximum rotation (reached for $\nu \simeq \nu_0 - 0.9\theta$) and maximum circular dichroism of the band is as follows:

$$\Phi = \frac{100(\epsilon_l - \epsilon_r)}{2.85}$$

where Φ is in radians.

Since the following relation holds between circular dichroism and ellipticity $(\epsilon_l - \epsilon_r) = \frac{4\varphi \log_{10} e}{cl}$, there is obtained $\alpha_{\max.} \simeq 0.61\varphi_{\max.}$, $\alpha_{\max.}$ being the maximum rotation and $\varphi_{\max.}$ the maximum ellipticity for a given band. Drude's formula led to the relation already mentioned $\alpha_{\max.} \simeq 0.5\varphi_{\max.}$

If the curves of the rotatory dispersion calculated by the complete Drude formula and by Kuhn's formula (11) are compared, it becomes apparent that for values approaching $\nu = \nu_0$ the rotation increases more rapidly with Kuhn's equation than with Drude's.¹⁶ The experimental evidence is in favor of Kuhn's formula.

Lowry and Hudson¹⁷ found that absorption bands are, as a rule, symmetrical on a scale of wave lengths and not on a scale of frequencies; consequently the exponential equation representing the absorption curve fits the experimental data better if λ is the variable instead of ν .

Making this transformation, the equation of Kuhn (11) becomes

$$[M] = \frac{\Phi}{m} \frac{\lambda_\phi}{\lambda} \left[e^{-\left(\frac{\lambda - \lambda_0}{\theta}\right)^2} \int_0^{\frac{\lambda - \lambda_0}{\theta}} e^{x^2} dx + \frac{\theta}{2(\lambda + \lambda_0)} \right]$$

where m is the maximum value of the term inside the bracket; for most of the bands so far studied, $m \simeq 0.56$.

Distance of Coupled Electrons. Using the model discussed hitherto for two coupled oscillators the vibrations of which are at 90° from each other and separated by the distance d , Kuhn¹⁸ derived the equation

¹⁶ Levene and Rothen, *J. Chem. Phys.*, **2**, 681 (1934).

¹⁷ Lowry and Hudson, *Trans. Roy. Soc. (London)*, **A 232**, 117 (1933).

¹⁸ Kuhn and Bein, *Z. physik. Chem.*, **B 22**, 406 (1933).

$g \simeq \frac{2\pi d}{\lambda}$ for the maximum anisotropy factor under conditions of random distribution of molecules with respect to incident light. It was then found that in many instances the values of d were far greater than the molecular dimensions. Theoretical investigation showed that maximum dichroism is indeed to be expected when the two vibrations are oriented at 90° ; the anisotropy factor, on the other hand, is maximum when the two vibrations are nearly anti-parallel, in other words, when the system is a quadrupole. (See Fig. 6.)

For a random distribution, $d \geq \frac{\sqrt{3}}{4\pi} g_0 \lambda_0 \sqrt{f}$, where the constants have already been defined.

Calculations showed that in every case investigated the values of d did not extend over the molecular dimensions. However, these values do not give any real information as to the actual distance d since they are only minimum values.

Application of the Simple Drude Formula $\alpha = \sum \frac{A_i}{\lambda^2 - \lambda_i^2}$ for the Interpretation of the Rotatory Dispersion of the Organic Molecules in a Region of Transparency. It should

be emphasized once more that the Drude formula expresses the course of rotatory dispersion if the condition $\sum A_i = 0$ is fulfilled. However, this does not mean that the sign of the partial rotations alternates regularly in the order of the λ_i in which $\lambda_1 > \lambda_2 \dots > \lambda_i$; it means only that there is an equal number of positive and negative terms, and that their sum is zero.

It was the merit of Lowry and his students to demonstrate that the dispersion curves of organic substances may be expressed by such formulas, and Tschugaeff was the first to emphasize for organic compounds the relation of the dispersion to the absorption regions of the

molecules. Lowry observed that in certain cases a single-term Drude formula was sufficient, in others, a formula consisting of two or more

* Kuhn and Bein, *Z. physik. Chem.*, **B 22**, 406 (1933). (Courtesy of publishers.)

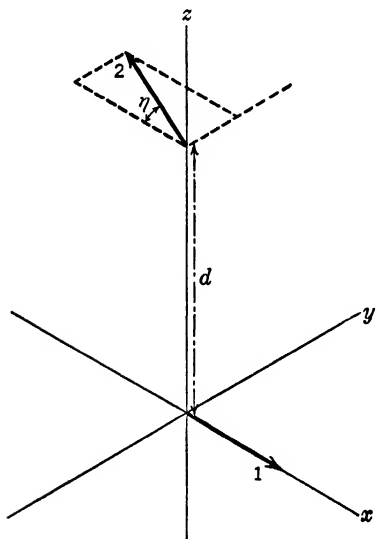


FIG. 6*

terms was required to represent the rotatory dispersion curves. It is advantageous to express the rotatory dispersion curves graphically by plotting $1/\alpha$ against λ^2 , as was originally done by Biot. A straight line is obtained when the dispersion can be expressed by a single term. Lowry called this type of dispersion "simple."^{*} He calls the dispersion "complex" when the function $1/\alpha = f(\lambda^2)$ deviates from linearity, which means that not less than two terms are required to express the dispersion curve. Among the "complex" rotatory dispersion curves, those are termed "anomalous" which exhibit an inflection, a maximum, and a reversal of sign of rotation with decreasing wave lengths. It should be emphasized that in dispersion curves "anomalies" which lie outside absorption bands are produced by a mechanism entirely different from those previously discussed in connection with the "Cotton effect."

It will be shown presently that a study of the curve $1/[M] = f(\lambda^2)$ may dissect the molecular rotation into two partial components, one corresponding to the partial rotation of the band with the longest wave length, the other corresponding to the sum of the remaining partial rotations. The analysis may first be attempted graphically, but as the deviation of $1/[M] = f(\lambda^2)$ from linearity is usually very small, an algebraic analysis has to be resorted to.¹⁹

If $1/[M] = f(\lambda^2)$ is not a linear function, it may be supposed as a first approximation that the dispersion can be expressed by two simple Drude terms. The precision attained in most measurements does not necessitate the calculation of a three-term formula.

The curvature of the graph $(1/\alpha, \lambda^2)$ varies in a definite manner, depending upon the relation between the terms $\frac{A_1}{\lambda^2 - \lambda_1^2}$ and $\frac{A_2}{\lambda^2 - \lambda_2^2}$. Evidently for very small intervals of the dispersion curve it is possible to find an expression $A_0/(\lambda^2 - \lambda_0^2)$ which very nearly satisfies the right-hand term of the expression,

$$[M] = A_1/(\lambda^2 - \lambda_1^2) \pm A_2/(\lambda^2 - \lambda_2^2)$$

The value of λ_0 and its variation with successive small wave-length intervals provides the required information on the relative values of the four constants of the two Drude term formulas.

^{*} In the single-term Drude formula, $\alpha = \frac{A}{\lambda^2 - \lambda_0^2}$, the empirical dispersion constant λ_0 acquires a physical meaning only when the formula expresses the entire experimental dispersion curve close to the region of absorption of the active band.

¹⁹ Hunter, *J. Chem. Soc.*, **125**, 1198 (1924).

The following four combinations have to be considered:
Two terms of the same sign (for long wave lengths).

$$(1) \quad \lambda_1 > \lambda_2, \quad A_1/(\lambda^2 - \lambda_1^2) > A_2/(\lambda^2 - \lambda_2^2)$$

$$(2) \quad \lambda_1 > \lambda_2, \quad A_1/(\lambda^2 - \lambda_1^2) < A_2/(\lambda^2 - \lambda_2^2)$$

Two terms of opposite sign (for long wave lengths).

$$(3) \quad \lambda_1 > \lambda_2, \quad A_1/(\lambda^2 - \lambda_1^2) > A_2/(\lambda^2 - \lambda_2^2)$$

$$(4) \quad \lambda_1 > \lambda_2, \quad A_1/(\lambda^2 - \lambda_1^2) < A_2/(\lambda^2 - \lambda_2^2)$$

Case 1. The resulting curve is practically a straight line for the long wave lengths, the curvature becoming more pronounced when λ approaches λ_1 . The values of λ_0 lie in between λ_1 and λ_2 ($\lambda_2 < \lambda_0 < \lambda_1$) and for smaller wave lengths are progressively shifted towards λ_1 .

Case 2. This case is similar to 1 but the curvature is accentuated. The displacements of λ_0 are greater, but the condition $\lambda_2 < \lambda_0 < \lambda_1$ still holds.

Case 3. Here the condition is $\lambda_2 < \lambda_1 < \lambda_0$. With decreasing wave lengths there is a displacement of λ_0 towards λ_1 . The curve generally deviates from a straight line more than in cases 1 and 2. The apparent shift of λ_0 may be considerable when the whole spectrum is considered. Rotations in the visible region may lead to λ_0 values 1500 Å higher than those obtained from rotations in the ultra-violet. (This effect is more pronounced the more nearly A_1 and A_2 approach the same magnitude.)

Case 4. With decreasing wave lengths, the curve first decreases to a minimum at λ_m ($\lambda_2 < \lambda_1 < \lambda_m$), then increases to $+\infty$, reappears at $-\infty$, and approaches zero for the smaller wave lengths. (The rotation goes through a maximum, decreases, reaches a zero value, and increases in the opposite direction.) The λ_0 values calculated from measurements in the long-wave-length region are smaller than λ_2 ($\lambda_0 < \lambda_2 < \lambda_1$) and become negative for λ values approaching λ_m . *This last case corresponds to an anomalous dispersion in a region outside an absorption band.*

From this analysis it follows that the graph $(1/\alpha, \lambda^2)$ leads to information about the direction of rotation of each of the two contributions and their relative values.

For a long time it was thought that a mixture of two kinds of active molecules of opposite sign with different dispersion ratios was a necessary condition for the obtaining of anomalous dispersion. Anomalies of this

type were exhaustively investigated by Biot, and it is noteworthy that even up to recent date the anomalous dispersion of tartaric acid was interpreted on the assumption of two dynamic isomers by authorities such as Lowry, notwithstanding the fact that Bruhat had emphasized that this assumption was superfluous.

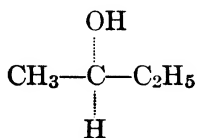
Tschugaeff was the first to state clearly the three following possible causes for anomalous dispersion in a region of transparency:

- (1) The two partial rotations correspond to two different molecular species.
- (2) The two partial rotations have their origin in two active (or asymmetric) centers belonging to the same molecule.
- (3) The two partial rotations have their origin in groups attached to a single asymmetric carbon atom.

Absolute Configuration. Kuhn²⁰ attempted to determine the absolute configuration of the simplest secondary carbinol, methylethylcarbinol. It was assumed that (1) the CH₃ and C₂H₅ radicals, as well as the asymmetric carbon atom, can be considered as isotropic resonators; (2) the OH group is anisotropic, for, as a second approximation, its anisotropy is a necessary condition for the appearance of a rotatory contribution—if the OH group were isotropic, optical activity would disappear for an approximation of the same order; (3) there is a restricted rotation of the group OH around the C–O axis.

The calculation consisted in identifying a definite vibration of the OH group with an absorption band of the same group.

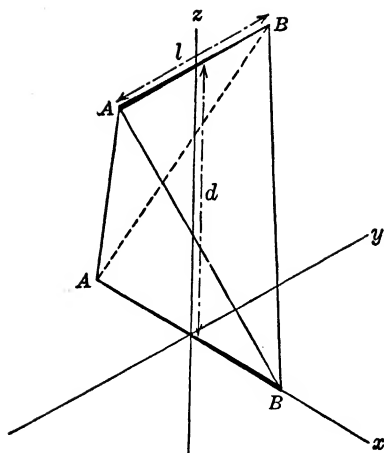
From measurements of Kerr effect, and assuming that the partial contribution of the first band is predominant, Kuhn came to the conclusion that the carbinol with the following configuration is levorotatory: *



Many arguments seriously limit this conclusion. From measurements on a series of secondary carbinols, it has been found that the sign of the first rotatory contribution of secondary carbinols of the above type is opposite to the sense of the rotation observed in the visible part of the spectrum; hence the above carbinol should be dextrorotatory, a conclusion arrived at by the theory of Boys.

²⁰ Kuhn, *Z. physik. Chem.*, **B 31**, 23 (1936).

* The dotted lines go to groups below the plane of the paper and the full lines to groups above the plane of the paper.

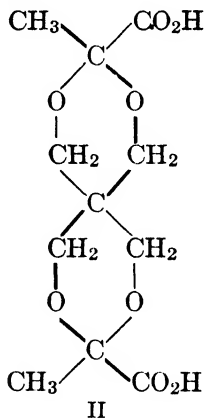
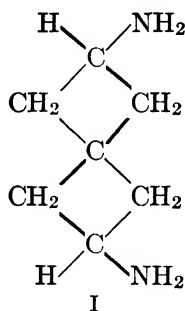


Dextro rotation
when
 $\nu_A < \nu_B$

FIG. 7*

Born's Newest Development.

Born²¹ made a new attempt to obtain an equation connecting optical activity with some known parameters of the molecule. His model (Fig. 7) consists of two pairs of resonators. By perturbation methods calculation was made of the mutual reaction of the two pairs of resonators, as well as the mutual reaction of the two resonators in each pair. Born's general expression is greatly simplified when the model has a binary axis of symmetry and when the two pairs of vibrators are perpendicular to each other. (See Fig. 7.) Active molecules (I and II) of such type are known.



Born's formula for such simplified models, as rewritten by Lowry, is

$$\alpha = 3.49 \cdot 10^{-11} \frac{\rho(n^2+2)}{M} f_1^2 f_2^2 \frac{\lambda_1^6 \lambda_2^6}{(\lambda_2^2 - \lambda_1^2)^3} \left[\frac{\lambda_1^2}{\lambda^2 - \lambda_1^2} - \frac{\lambda_2^2}{\lambda^2 - \lambda_2^2} \right] \frac{1}{l^8} F(\xi)$$

where

$$F(\xi) = \frac{\xi(\xi^2 - \frac{1}{2})^2}{(\xi^2 + \frac{1}{2})^6}, \quad \xi = \frac{d}{l}$$

d = distance between the two pairs of vibrators in Ångström units.

²¹ Born, *Proc. Roy. Soc. (London)*, **A 150**, 84 (1935).

* Born, *Proc. Roy. Soc. (London)*, **A 150**, 84 (1935). (Courtesy of publishers.)

l = distance between the vibrators in Ångström units.

α = rotation in degrees per decimeter.

ρ = density.

n = refractive index.

M = molecular weight of the substance.

λ_1, λ_2 = wave length in Ångström units of the natural vibrations of the two different resonators (NH_2 and H in the example chosen by Born).

f_1, f_2 = the intensity factors of the two vibrations as defined previously.

The formula is valid only in a region of transparency and represents the sum of two contributions.

Inasmuch as the formula gives a relation between the sign of rotation and the spatial distribution of the vibrators, the absolute configuration of the model is determined.

Born assigns a dextrorotation to the above model (I); the same relation was obtained by Kuhn for dipyrucic erythritol (II).

PART II. APPLICATION OF ROTATORY DISPERSION MEASUREMENTS TO THE CORRELATION OF CHEMICAL STRUCTURE AND OPTICAL ACTIVITY

The principal interest of organic chemistry in the correlation of chemical structure and optical activity lies in the search for connections between chemical structure and optical activity of substances with known configurations. These connections would permit the correlation of the configuration of substances in cases where classical organic chemistry furnishes no adequate method. Such information, if obtained, might, in its turn, serve to elucidate the mechanism of certain organic reactions.

Partial Rotations in Homologous Series

From the discussion of the physical theories of optical rotation, it is evident that, in order to obtain adequate information with regard to relationship between structure and rotation, it is necessary to compare not the rotation of the molecule as a whole but the partial rotations of the chromophoric groups. The chemical literature of the past offers comparatively little material for such comparisons because organic chemists were not aware of the importance of such investigations. Another reason is the lack, until recent years, of suitable instruments and of suitable material on which such studies could be carried out. Indeed, in the opening address of the symposium on "Optical Rotatory Power" of the Faraday Society in 1914, Armstrong²² states, "In the

²² Armstrong, *Trans. Faraday Soc.*, **10**, 44 (1914).

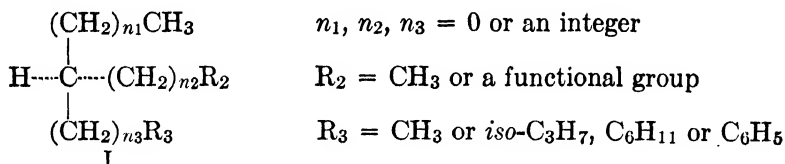
past, apparently, workers have dealt too much with substances such as tartrates which presumably can occur in more than one form. In the future we must deal more with substances of *known configuration* which are not subject to change, *if there be such*—if we are to solve problems such as have been stated by Crum Brown and by Guye and correlate rotatory power with structure."

No one at the symposium of 1914 pointed out the significance of the partial rotation. Yet that concept was even then not new. Drude, Cotton, and Tschugaëff had emphasized its significance, but little application of it in organic chemistry was made save by Lowry, until the appearance of the publication by W. Kuhn in 1930.

The present chapter will be limited to the discussion of only those investigations which meet the following requirements:

1. The active substances have one asymmetric carbon atom.
2. The rotatory dispersion curves are known over a wave-length interval broad enough to permit an estimate of the partial rotations.

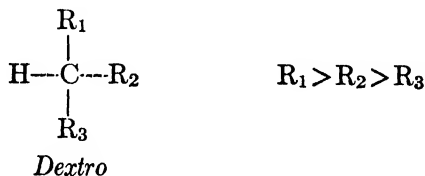
All the substances to be discussed can be expressed by the general formula,



NORMAL ALIPHATIC SERIES

All substances to be discussed in this chapter can be regarded as derived from the normal aliphatic hydrocarbons and alcohols, by substitution of R_2 or R_3 . Hence the dispersion curves of these latter substances may likewise be regarded as reference curves.

Hydrocarbons. The substances of this group may be regarded as the most homogeneous from the viewpoint of their chromophoric properties. The asymmetry of the molecules is produced exclusively by the differences in the length of the carbon chains in the individual groups. The systems of the absorption bands of the radicals are likewise conditioned by the differences in the length of the chain, and are therefore closely analogous.



When the dispersion curve of a hydrocarbon²³ of the above type is expressed by a one-term Drude formula, the dispersion constant found ($\lambda_0 \simeq 1600$ to 1700 \AA) is nearer to the visible region than the first absorption band of an alkyl group. It may then be inferred that the rotatory dispersion curve is the resultant of two rotatory components of opposite sign, the rotation in the visible region being determined by the heaviest group. Indeed, when $R_2 > R_1$, the rotation of the above hydrocarbon is *levo*.

Alcohols. Interest in the optical activity of alcohols was stimulated by the work of Pickard and Kenyon,²⁴ who in 1911 developed a method for the resolution of secondary carbinols and thus made available for investigation a large group of synthetic substances.

Rotatory dispersion measurements, taken first in the visible region and recently extended into the ultra-violet,²⁵ have shown that the distance of the hydroxyl group from the asymmetric carbon atom, as well as the structure of the alkyl groups of the rest of the molecule, produced marked effects on the dispersive power of the alcohols. For all alcohols, the first absorption region ($\lambda \simeq 1800 \text{ \AA}$) seems to possess very little or no activity. For example, the high dispersion constant found for amyl alcohol ($\lambda_0 \simeq 1850 \text{ \AA}$) for a one-term formula from measurements in the visible region does not mean that the first absorption region is active, as can be seen from the dispersion in the ultra-violet. (See p. 1800, Case 3.)

To Pickard and Kenyon²⁶ belongs also the credit for first observing anomalous dispersion in simple substances with one asymmetric center.*

Most striking were the observations on the rotatory dispersion of *dextro*-octanol-4 (propylbutylcarbinol). It was known that this carbinol rotated in the same direction as the lower members homologous with respect to n_1 but was levorotatory in ether.²⁵

As previously stated, such a phenomenon indicated anomalous dispersion in one of the two states. The analysis of the dispersion curves disclosed that the dispersion was normal in ether and anomalous in the homogeneous state. Therefore the first active absorption region of this *dextro*-carbinol furnishes a levorotatory partial contribution. Since in

²³ Levene and Rothen, unpublished results.

²⁴ Pickard and Kenyon, *J. Chem. Soc.*, **99**, 45 (1911).

²⁵ Levene and Rothen, *J. Biol. Chem.*, **116**, 209 (1936).

²⁶ Pickard and Kenyon, *J. Chem. Soc.*, **105**, 830 (1914).

* So strong at the time was the belief that the cause of anomalous dispersion was the presence in the medium of two molecular species that Pickard and Kenyon gave the following explanation to their observations: "The specific rotatory powers of these (esters) can be correlated on the assumption that each ester is a mixture of two isomerides which have rotatory powers of opposite sign and different dispersive powers."

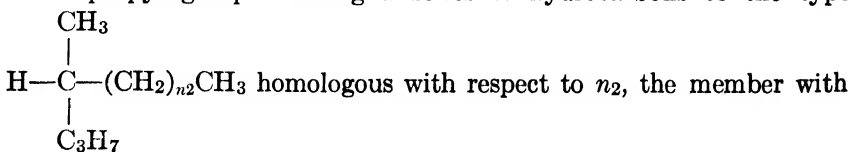
homologous series the direction of rotation of corresponding chromophoric regions remains unchanged, the dispersion of the lower homologous members must also be anomalous, but their first partial rotation must be small compared to the partial rotation associated with the absorption bands of higher frequencies. Other facts also substantiate this conclusion.

Kenyon and Barnes²⁷ prepared a series of ethers from *dextro*-nonanol-3 (from methyl up to nonyl ether). With the exception of the methyl ether, all the ethers were dextrorotatory. The *dextro* ethers exhibited typical anomalous dispersion, whereas the dispersion of the methyl ether was normal, the two corresponding partial contributions being of the same sign as in the higher ethers. It is legitimate to assume that what holds for the ethers also holds for the corresponding carbinols, inasmuch as the substitution of an alkyl group for a hydrogen atom does not introduce into the molecule a group having an absorption band in the longer-wave-length region.

INFLUENCE OF CHROMOPHORIC GROUPS OF NON-POLAR CHARACTER (EFFECT OF DISTANCE FROM THE ASYMMETRIC CENTER)

In the present section will be discussed the effect brought about when R_2 represents an isopropyl, phenyl, cyclohexyl, or ethylenic group. Such substances offer an opportunity to compare the partial rotation of the chromophoric group as a function of n_2 . The change of the rotation with the increase of the distance of the chromophoric group from the asymmetric carbon atom is a striking phenomenon. This effect has been pointed out by Tschugaeff²⁸ and by Rupe,²⁹ but all they noted was a gradual rise or drop in the rotation of consecutive members until a constant value was finally reached.

Effect of the Isopropyl Group.³⁰ The effect of a group on the total molecular rotation is two-fold: on the one hand, it introduces a partial rotation of its own; on the other hand, by its vicinal influence it may change the partial rotation of the other groups. The simplest change that can be produced in a hydrocarbon is the substitution of an isopropyl for a propyl group. Taking a series of hydrocarbons of the type



²⁷ Kenyon and Barnes, *ibid.*, **125**, 1395 (1924).

²⁸ Tschugaeff, *Trans. Faraday Soc.*, **10**, 70 (1914).

²⁹ Rupe, *ibid.*, **10**, 46 (1914).

³⁰ Levene and Rothen, *J. Org. Chem.*, **1**, 76 (1936).

$n_2 = 2$ has a zero rotation, and the direction of the member having $n_2 = 3$ is of opposite sign to that of the first member. Yet in all members the rotatory dispersion remains normal. If, however, the isopropyl group is substituted for the propyl, all members of the new series rotate in the direction opposite to that of the lowest members of the normal series, the reason being that in the first series the direction of rotation is determined principally by the clockwise or counterclockwise arrangement of the groups according to their volume, whereas in the second some other factors influence the direction of rotation (Table I). In the case of hydrocarbons there may be reason to assume that the direction of rotation in the visible region is actually due to the partial rotation of the isopropyl group.

In the secondary alcohols³⁰ the substitution of an isopropyl for a propyl group produces a similar effect on the rotation in the visible region. From Table II it can be seen that a rotatory contribution of opposite sign to the rotation of the lower members of the propyl series is introduced in the isopropyl alcohols. The rotatory dispersion of the members of the two groups of substances likewise seems very similar. Thus, for methylisopropylpropylmethane, $\lambda_0 = 1700 \text{ \AA}$ (obtained by a one-term Drude formula) which is similar to the λ_0 values obtained for normal aliphatic hydrocarbons. The dispersion constants calculated by a one-term Drude formula from measurements in the visible region are $\lambda_0 = 1820 \text{ \AA}$ for methylisopropylcarbinol, and $\lambda_0 = 1650 \text{ \AA}$ for propylisopropylcarbinol.²³ Two Drude terms of opposite sign (p. 1800, Case 3) have to be used to represent the rotary dispersion of these two substances from the visible down to $\lambda = 2400 \text{ \AA}$. The dispersion constant of the first term is the same for both compounds ($\lambda_1 \simeq 1480 \text{ \AA}$). Whether the effect of the isopropyl group in these alcohols is due to its vicinal effect or to its own partial contribution, cannot be stated definitely as yet. However, as will be seen later, the effect of the isopropyl group in carboxylic acids is definitely vicinal.

The vicinal action of the isopropyl group is particularly striking in 1-bromo-2,3-dimethylbutane (methylisopropylbromoethane) and the corresponding iodo derivative.²³ These compounds show a normal dispersion, whereas the course of the dispersion is anomalous in the corresponding members of the normal series. The dispersion constant of methylisopropylbromoethane ($\lambda_0 = 1800 \text{ \AA}$) indicates that the absorption region of the bromine conditions the rotation in the visible, whereas in the normal series the corresponding band furnishes a partial rotation of opposite direction from that of the rotation in the visible region. The value of λ_0 ($\lambda_0 \simeq 1900 \text{ \AA}$) for the iodo derivative shows that the first absorption band furnishes a negligible contribution. Thus

TABLE I
CONFIGURATIONALLY RELATED HYDROCARBONS CONTAINING AN ISOPROPYL, ISOBUTYL, OR ISOAMYL GROUP
[M_D^{25} max. (homogeneous)]

Series	$-\text{CH} \begin{array}{c} \text{CH}_3 \\ \diagup \quad \diagdown \\ \text{CH}_2 \end{array}$ Series	$-(\text{CH}_2)_3\text{CH}_3$ Series	$-\text{CH}_2-\text{CH} \begin{array}{c} \text{CH}_3 \\ \diagup \quad \diagdown \\ \text{CH}_3 \end{array}$ Series	$-(\text{CH}_2)_4\text{CH}_3$ Series	$-(\text{CH}_2)_5-\text{CH} \begin{array}{c} \text{CH}_3 \\ \diagup \quad \diagdown \\ \text{CH}_3 \end{array}$ Series
$-(\text{CH}_2)_2\text{CH}_3$ Series CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_2\text{H}_5$ C_3H_7 (n) -9.9	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_2\text{H}_5$ C_3H_7 (iso) +26.4 *	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_2\text{H}_5$ C_4H_9 (n) -11.4	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_2\text{H}_5$ C_4H_9 (iso) -21.3	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_2\text{H}_5$ C_5H_{11} (n) -12.0	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_2\text{H}_5$ C_5H_{11} (iso) -11.9
CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_3\text{H}_7$ (n) C_3H_7 (n) 0	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_3\text{H}_7$ (n) C_3H_7 (iso) +5.5 *	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_3\text{H}_7$ (n) C_4H_9 (n) -1.7	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_3\text{H}_7$ (n) C_4H_9 (iso) -14.9	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_3\text{H}_7$ (n) C_5H_{11} (n) -2.4	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_3\text{H}_7$ (n) C_5H_{11} (iso) -3.5
CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_4\text{H}_9$ (n) C_3H_7 (n) +1.7		CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_4\text{H}_9$ (n) C_4H_9 (n) 0	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_4\text{H}_9$ (n) C_4H_9 (iso) -11.9	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_4\text{H}_9$ (n) C_5H_{11} (n) -0.86	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_4\text{H}_9$ (n) C_5H_{11} (iso) -1.5
CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_5\text{H}_{11}$ (n) C_3H_7 (n) +2.4	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_5\text{H}_{11}$ (n) C_3H_7 (iso) +1.1 *	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_5\text{H}_{11}$ (n) C_4H_9 (n) +0.86	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_5\text{H}_{11}$ (n) C_4H_9 (iso) -9.3	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_5\text{H}_{11}$ (n) C_5H_{11} (n) 0	CH_3 $\text{H} \cdots \text{C} \cdots \text{C}_5\text{H}_{11}$ (n) C_5H_{11} (iso) -0.2

* Not maximum rotation.

TABLE II

SECONDARY CARBINOLS CONTAINING AN ISOPROPYL OR ISOBUTYL GROUP
 $[M]_D^{25}$ max. (homogeneous)

$-(CH_2)_2CH_3$	$\begin{array}{c} CH_3 \\ \\ -CH \\ \\ CH_3 \end{array}$	$-(CH_2)_2CH_3$	$\begin{array}{c} CH_3 \\ \\ -CH_2-CH \\ \\ CH_3 \end{array}$
$\begin{array}{c} CH_3 \\ \\ H \cdots C \cdots OH \\ \\ C_3H_7 \text{ (} n \text{)} \\ +12.1 \end{array}$	$\begin{array}{c} CH_3 \\ \\ H \cdots C \cdots OH \\ \\ C_3H_7 \text{ (iso)} \\ +4.7 \end{array}$	$\begin{array}{c} CH_3 \\ \\ H \cdots C \cdots OH \\ \\ C_4H_9 \text{ (} n \text{)} \\ +11.8 \end{array}$	$\begin{array}{c} CH_3 \\ \\ H \cdots C \cdots OH \\ \\ C_4H_9 \text{ (iso)} \\ +21.1 \text{ (?) } \end{array}$
$\begin{array}{c} C_2H_5 \\ \\ H \cdots C \cdots OH \\ \\ C_3H_7 \text{ (} n \text{)} \\ +4.2 \end{array}$	$\begin{array}{c} C_2H_5 \\ \\ H \cdots C \cdots OH \\ \\ C_3H_7 \text{ (iso)} \\ -16.7 \end{array}$	$\begin{array}{c} C_2H_5 \\ \\ H \cdots C \cdots OH \\ \\ C_4H_9 \text{ (} n \text{)} \\ +9.4 \end{array}$	$\begin{array}{c} C_2H_5 \\ \\ H \cdots C \cdots OH \\ \\ C_4H_9 \text{ (iso)} \\ +24.6 \end{array}$
$\begin{array}{c} C_3H_7 \text{ (} n \text{)} \\ \\ H \cdots C \cdots OH \\ \\ C_3H_7 \text{ (} n \text{)} \\ 0 \end{array}$	$\begin{array}{c} C_3H_7 \text{ (} n \text{)} \\ \\ H \cdots C \cdots OH \\ \\ C_3H_7 \text{ (iso)} \\ -27.1 \end{array}$	$\begin{array}{c} C_3H_7 \text{ (} n \text{)} \\ \\ H \cdots C \cdots OH \\ \\ C_4H_9 \text{ (} n \text{)} \\ +0.95 \end{array}$	$\begin{array}{c} C_3H_7 \text{ (} n \text{)} \\ \\ H \cdots C \cdots OH \\ \\ C_4H_9 \text{ (iso)} \\ +16.3 \end{array}$
$\begin{array}{c} C_4H_9 \text{ (} n \text{)} \\ \\ H \cdots C \cdots OH \\ \\ C_3H_7 \text{ (} n \text{)} \\ -0.95 \end{array}$	$\begin{array}{c} C_4H_9 \text{ (} n \text{)} \\ \\ H \cdots C \cdots OH \\ \\ C_3H_7 \text{ (iso)} \\ -35.9 \end{array}$	$\begin{array}{c} C_4H_9 \text{ (} n \text{)} \\ \\ H \cdots C \cdots OH \\ \\ C_4H_9 \text{ (} n \text{)} \\ 0 \end{array}$	$\begin{array}{c} C_4H_9 \text{ (} n \text{)} \\ \\ H \cdots C \cdots OH \\ \\ C_4H_9 \text{ (iso)} \\ +13.8 \end{array}$
$\begin{array}{c} C_6H_{11} \text{ (} n \text{)} \\ \\ H \cdots C \cdots OH \\ \\ C_3H_7 \text{ (} n \text{)} \\ \textit{levo} \end{array}$	$\begin{array}{c} C_6H_{11} \text{ (} n \text{)} \\ \\ H \cdots C \cdots OH \\ \\ C_3H_7 \text{ (iso)} \\ -38.2 \end{array}$	$\begin{array}{c} C_6H_{11} \text{ (} n \text{)} \\ \\ H \cdots C \cdots OH \\ \\ C_4H_9 \text{ (} n \text{)} \\ \textit{levo} \end{array}$	$\begin{array}{c} C_6H_{11} \text{ (} n \text{)} \\ \\ H \cdots C \cdots OH \\ \\ C_4H_9 \text{ (iso)} \\ +11.3 \end{array}$

the events in the isopropyl series are significant in demonstrating how small changes in molecular structure may produce very significant effects on the anisotropy of the low frequency bands.

As can be seen from Table I, when R_3 is an isopropyl group, the rotation of the hydrocarbons homologous with respect to n_3 changes sign when n_3 is changed from 0 to 1. In both series the absolute rotations are enhanced by the substitution of an isopropyl group for a propyl group. When $n_3 = 2$ the specific effect of the isopropyl group is very small.

This conclusion is best illustrated by the fact that for methylamylisobutylmethane, $[M]_D^{25}$ max. = -0.2° . *The effect of the distance of the isopropyl group from the asymmetric center in carbinols is similar to that in hydrocarbons, as can be seen from Table II.*

The specific effect of the isopropyl group is exhausted at a comparatively short distance from the asymmetric center.

Effect of Phenyl and Cyclohexyl Groups. A great deal of very valuable work on the effect of the phenyl group has been done in the past by Haller, by Hilditch, and by Rupe. The earlier work was limited to the rotation in the visible spectrum and could yield no clue to the question of the partial rotations of the individual groups. An extensive investigation into the effects of substitution of a cyclohexyl or phenyl group for a hexyl group was made recently by Kuhn and Biller.³¹ The authors studied the dispersion curves of nitrites, acetates, carbomethoxy derivatives, and phthalates. It was found that the absorption band at $\lambda = 2600 \text{ \AA}$ was not anisotropic for most derivatives. A decision as to the vicinal effect of each of the three groups (C_6H_{13} , C_6H_{11} , and C_6H_5) was reached by Kuhn and Biller by a comparison of the rotations and the rotatory dispersions of several derivatives mentioned above. The results are presented in Table III. In all three series the shift of rotation on passing from the carbinols to the derivatives is in the same direction. Inasmuch as the configurations of two of the three carbinols had been correlated by Levene and Stevens,³² the authors concluded that the three radicals exerted a similar vicinal effect, and that hexylcyclohexylcarbinol should be optically inactive. They suggested further that the partial rotations of the hexyl, cyclohexyl, and phenyl groups have the same sign.

However, recent results do not fall completely in line with the conclusions of Kuhn and Biller. It may be stated here that observations limited to a single member of a homologous series do not always reveal the whole truth about the partial contribution of each individual chromophoric group, particularly when the active absorption bands are located in the more distant ultra-violet region. The rotations of the members of the homologous series of cyclohexyl carbinols (see Table IV) bear a striking resemblance to those of the members of the isopropyl series. It may be assumed, therefore, that hexylcyclohexylcarbinol will be optically active, and not inactive, as predicted by Kuhn and Biller. True, the two predictions have not, as yet, been tested experimentally. However, Levene and Harris³³ have shown that methyl-*n*-octyl- β -cyclohexyl-

³¹ Kuhn and Biller, *Z. physik. Chem.*, **B 29**, 1 (1935).

³² Levene and Stevens, *J. Biol. Chem.*, **89**, 471 (1930).

³³ Levene and Harris, *ibid.*, **111**, 735 (1935).

ethyl-methane has a maximum molecular rotation $[M]_D \simeq 5.2$. In the isopropyl hydrocarbon having $n_3 = 2$, (2,5-dimethyldecane) $[M]_D = -0.2^\circ$, as was stated earlier.

TABLE III

$[M]_D^{20}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H}-\text{C}-\text{OH} \\ \\ \text{R} \end{array}$	R	Alcohol	Phthalic Ester	Carbo-methoxy Derivative	Nitrite	Acetate
Methyl- <i>n</i> -hexylcarbinol. . .		C_6H_{13}	+12.7	+133.7	+8.8	+11.0
Methylcyclohexylcarbinol..		C_6H_{11}	+7.4	+150.0	-6.1	-9.8	-5.4
Methylphenylcarbinol.....		C_6H_5	-52.0	+110.0	-169.7	-75.3	-183.0

TABLE IV

MAXIMUM MOLECULAR ROTATIONS OF CYCLOHEXYLCARBINOLS $[M]_D^{25}$

$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{C}_6\text{H}_{11} \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{C}_6\text{H}_{11} \end{array}$	$\begin{array}{c} \text{C}_3\text{H}_7 (n) \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{C}_6\text{H}_{11} \end{array}$	$\begin{array}{c} \text{C}_4\text{H}_9 (n) \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{C}_6\text{H}_{11} \end{array}$
+6.5	-11.5	-17.0	-21.9

The effect of the phenyl group in the secondary carbinols resembles that of the cyclohexyl group, except that it is more pronounced. (See Table V.) The probability is that the partial rotation of the phenyl group, rather than that of the hydroxyl group, determines the rotation of the carbinols in the visible region. The λ_0 value found from rotatory dispersion measurements ($\lambda_0 \simeq 2070 \text{ \AA}$) for methylphenylcarbinol makes it improbable that the rotation in the visible region is the resultant of the partial contribution of the hydroxyl group. The periodicity in the shift of rotation on the progressive increase of n_3 is also in agreement with this view. The dispersion of alcohols containing a phenyl group illustrates the difficulty encountered in interpreting the meaning of a dispersion curve when the two significant chromophoric groups possess absorption bands of approximately the same frequency, located at an appreciable distance from the visible region.

Conclusion. A comparison of observations on the substitution of an isopropyl group for a propyl, or of a cyclohexyl or a phenyl group for a hexyl, reveals that the effects of the three substituents are similar.

The Influence of an Ethylenic Linkage. The state of affairs on this question until 1914 is best summarized by Rupe²⁹ in his address at the

TABLE V
CONFIGURATIONALLY RELATED SECONDARY CARBINOLS AND TRISUBSTITUTED METHANES
[M]_D²⁵ max. (homogeneous)

$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{C}_6\text{H}_{13} \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{C}_6\text{H}_{11} \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_5 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_{13} \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_{11} \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_5 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_{13} \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_{11} \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_5 \end{array}$	+11.6
-11.5	-39.7	+10.6	+42.0	+51.7	+10.7	+10.0	+31.0		

symposium of the Faraday Society by the statement, "The presence of unsaturation leads to an irregularity in the rotatory effect, and not necessarily to an increased rotation."

The situation remained unaltered until recent years when a sufficient number of observations had accumulated on substances with one asymmetric center having the ethylenic group directly connected with the asymmetric carbon.

The rotations of a number of unsaturated carbinols and the corresponding saturated carbinols are given in Table VI^{33, 34}. The significant feature of the table is that the rotations of the unsaturated carbinols are opposite in sign to those of the saturated carbinols in all cases in which the ethylenic group is situated in the smaller alkyl group, but the rotation remains of the same sign when the ethylenic group is situated in the larger alkyl group.

This change in the rotation of the molecule as a whole is associated with the change of the partial rotation of the ethylenic group. This conclusion emerges clearly from analysis of the rotatory dispersion curves of these substances, which, by virtue of the positions of their chromophoric group, permits one to extend the observations to sufficient proximity to the absorption band and thus to obtain definite information as to the direction of the partial rotation of the double bond.

TABLE VI
CONFIGURATIONALLY RELATED CARBINOLS CONTAINING A DOUBLE BOND
[M]_D²⁵ max. (homogeneous)

$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{CH} \\ \\ \text{CH}_2 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{CH} \\ \\ \text{CH}_2 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{CH} \\ \\ \text{CH} \\ \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{CH} \\ \\ \text{CH} \\ \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{CH}_2 \\ \\ \text{CH} \\ \\ \text{CH}_2 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ (\text{CH}_2)_2 \\ \\ \text{CH} \\ \\ \text{CH}_2 \end{array}$
+28.2	+28.5	+9.8	<i>dextro</i>	+4.7	+17.2
↓	↓	↓	↓	↙	↓
$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{C}_2\text{H}_5 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{C}_2\text{H}_5 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{C}_2\text{H}_5 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{C}_2\text{H}_5 \end{array}$		$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{OH} \\ \\ \text{C}_2\text{H}_5 \end{array}$
-4.2	-9.4	-0.95	+12.1		+11.8

³⁴ Levene and Haller, *ibid.*, **83**, 579 (1929).

INFLUENCE OF CHROMOPHORIC GROUPS OF POLAR CHARACTER (R_2 IN THE GENERAL FORMULA)

[EFFECT OF DISTANCE FROM THE ASYMMETRIC CENTER (VALUE OF n_2)]

Two categories of substances will be discussed in this section. To the first category belong substances in which the configurations of all members homologous with respect to n_2 can be correlated by methods of

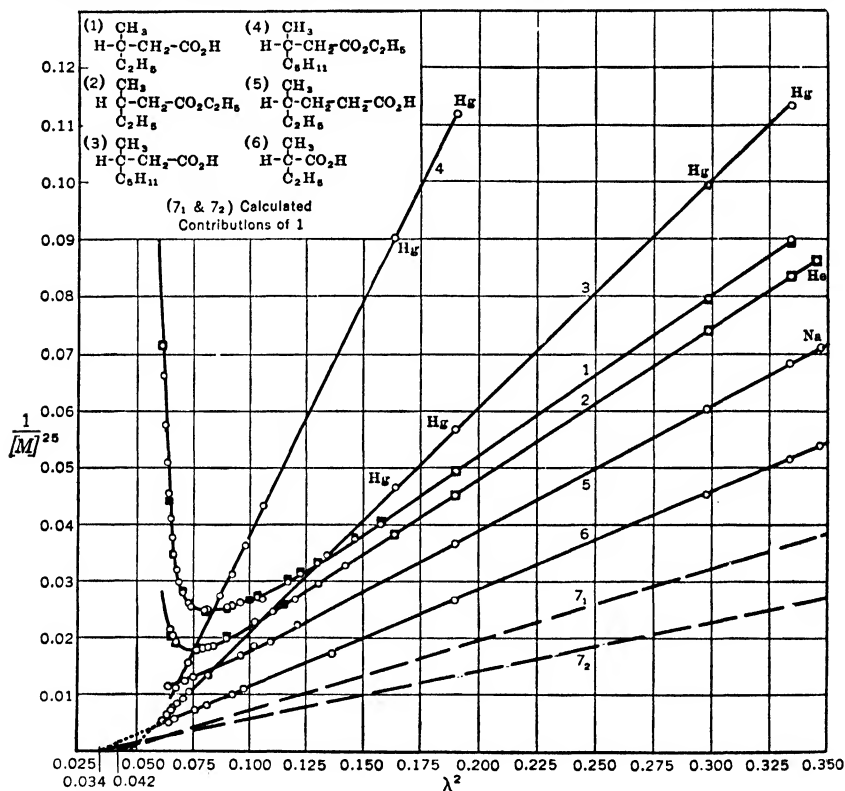


FIG. 8.*—Examples of the most characteristic dispersion curves of aliphatic acids and esters. Circles represent experimental values; solid squares, calculated values.

classical organic chemistry; to the second belong those whose configurations can be correlated only when $n_2 > 0$.

First Category. The changes in direction of the partial rotations of chromophoric groups with the increase of $n_2 = 0$ to $n_2 = 1$, cannot be explained on the basis of any theoretical models, even the most recent ones, advanced for the explanation of the mechanism of optical rotation.

* Levene, Rothen, and Marker, *J. Chem. Phys.*, **1**, 662 (1933). (Courtesy of publishers.)

TABLE VII
CONFIGURATIONALLY RELATED ALIPHATIC ACIDS CONTAINING A METHYL GROUP ON THE ASYMMETRIC CARBON ATOM
[M]_D²⁵ max. (homogeneous)

$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{CO}_2\text{H} \\ \\ \text{C}_2\text{H}_5 \end{array}$ <p style="text-align: center;">+18.0</p>	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{CH}_2\text{CO}_2\text{H} \\ \\ \text{C}_2\text{H}_5 \end{array}$ <p style="text-align: center;">+10.4</p>	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_2\text{CO}_2\text{H} \\ \\ \text{C}_2\text{H}_5 \end{array}$ <p style="text-align: center;">+13.6</p>	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_3\text{CO}_2\text{H} \\ \\ \text{C}_2\text{H}_5 \end{array}$ <p style="text-align: center;">+11.1</p>	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_4\text{CO}_2\text{H} \\ \\ \text{C}_2\text{H}_5 \end{array}$ <p style="text-align: center;">+12.2</p>
$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{CO}_2\text{H} \\ \\ \text{C}_2\text{H}_7 (n) \end{array}$ <p style="text-align: center;">+21.4</p>	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{CH}_2\text{CO}_2\text{H} \\ \\ \text{C}_2\text{H}_7 (n) \end{array}$ <p style="text-align: center;">-3.6</p>	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_2\text{CO}_2\text{H} \\ \\ \text{C}_2\text{H}_7 (n) \end{array}$ <p style="text-align: center;">+6.9</p>	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_3\text{CO}_2\text{H} \\ \\ \text{C}_2\text{H}_7 (n) \end{array}$ <p style="text-align: center;">+3.7</p>	

In many instances there is observed a periodic change in the shift of rotation in the visible region with the progressive increase in the value of n_2 . In other cases the periodicity comes to light only when the partial rotation of the chromophoric group is considered.

*Aliphatic Carboxylic Acids.*³⁰ Table VII gives the rotations of several series of aliphatic acids, and their dispersion curves are represented in Fig. 8. The rotary dispersion curves of *disubstituted acetic acids* can be expressed, for all members, by a one-term Drude formula having $\lambda_0 = 1850 \text{ \AA}$. This value indicates that the first absorption band of the CO_2H group at 2050 \AA displays a negligible Cotton effect.

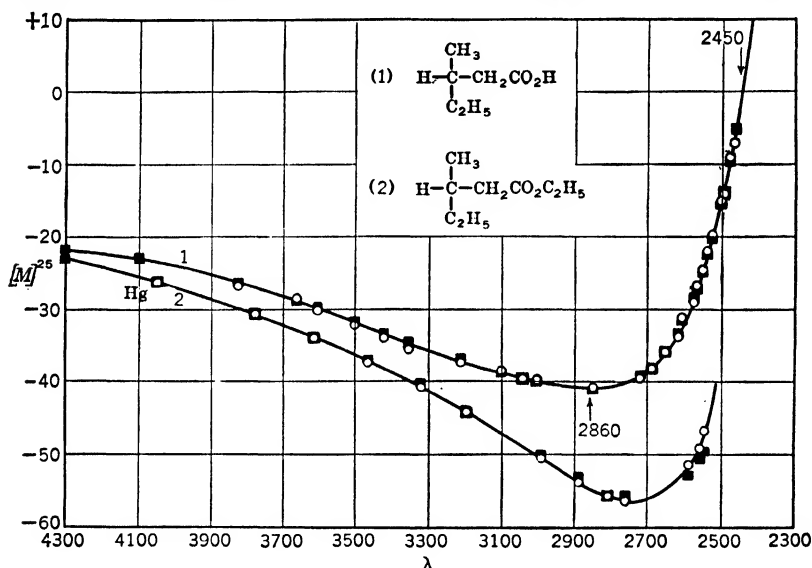


FIG. 9.*—Dispersions of two compounds exhibiting typical abnormality. Circles represent experimental values; solid squares, values calculated from a two-Drude-term formula.

*Disubstituted propionic acids*³⁰ ($n_2 = 1$) present a clear case of a series homologous with respect to n_3 in which the members differ as to the sign of their rotation in the visible region, but where the first and the second partial rotations remain of the same sign in all members of the series. Thus, the first member of the series is dextrorotatory, and its dispersion curve is anomalous, and can be expressed by the two-term Drude formula,

$$[M]^{25}_{\text{max.}} = -\frac{8.088}{\lambda^2 - 0.042} + \frac{11.63}{\lambda^2 - 0.034}$$

* Levene, Rothen, and Marker, *J. Chem. Phys.*, **1**, 662 (1933). (Courtesy of publishers.)

The other members have a rotation of opposite sign and exhibit a normal dispersion curve. Fig. 9 represents the dispersion of the first member of the series. In Table VIII are summarized the directions of the two partial rotations of the aliphatic carboxylic acids. The second partial rotation is probably furnished in the main by the carboxyl group. This consideration would lead to the conclusion that the sum of the two rotatory components of the carboxyl group changes sign on passing from the member having $n_2 = 0$ to that having $n_2 = 1$.

When the isopropyl group is substituted in methylpropylacetic acid for a propyl group, the resulting acid has the same sign of rotation, but the first absorption region ($\lambda \simeq 2100 \text{ \AA}$) which is inactive in the first acid has become active. The conditions are reversed in the substituted propionic acid series where the first band at $\lambda \simeq 2100 \text{ \AA}$ is active in the normal derivatives, whereas in the isopropyl series it is inactive and the dispersion of the acid is similar to that of the normal acetic acid series. The dispersion constants found for methylisobutylacetic acid

TABLE VIII *

ROTATION IN THE VISIBLE REGION AND PARTIAL ROTATIONS IN THE SERIES OF ALIPHATIC CARBOXYLIC ACIDS

Series	Rotation in Visible Region	Direction of Partial Rotation	
		2050 \AA	1850 \AA
Acetic	<i>dextro</i>	0	<i>dextro</i>
Propionic	<i>levo</i> (1st member excepted)	<i>levo</i>	<i>dextro</i>
Butyric	<i>dextro</i>	<i>levo</i>	<i>dextro</i>
Valeric	<i>dextro</i>	<i>levo</i>	<i>dextro</i>

* The value of the partial rotation of the first absorption band in the member with $n_2 = 0$ is given as zero. It is possible that this band may furnish a small partial rotation which is not accessible to measurement.

($\lambda_0 = 1730 \text{ \AA}$) and for methylisobutylpropionic acid ($\lambda_0 = 2100 \text{ \AA}$) show that these acids have the same dispersion as the corresponding acids of the normal series.

Phenyl Carboxylic Acids. The maximum molecular rotation of the acids of the aliphatic series is so small that their periodic variations as a function of n_2 may seem dubious when $n_2 > 1$. It is therefore important to note that a phenomenon, similar but much accentuated and clear beyond dispute, can be observed in acids containing a phenyl group. (See Table IX.)³⁰ In the phenyl acids, as in the secondary phenyl carbinols, the absorption bands at $\lambda \simeq 2600 \text{ \AA}$ are inactive.

TABLE IX
CARBOXYLIC ACIDS CONTAINING A PHENYL GROUP
[M_D^{25} max. (homogeneous) *]

	1st Part. Rot. + 2nd Part. Rot. -	1st Part. Rot. - 2nd Part. Rot. +	1st Part. Rot. - 2nd Part. Rot. +	1st Part. Rot. - 2nd Part. Rot. +
$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{CO}_2\text{H} \\ \\ \text{C}_6\text{H}_5 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{CO}_2\text{H} \\ \\ \text{C}_6\text{H}_5 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{CH}_2\text{CO}_2\text{H} \\ \\ \text{C}_6\text{H}_5 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_2\text{CO}_2\text{H} \\ \\ \text{C}_6\text{H}_5 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_3\text{CO}_2\text{H} \\ \\ \text{C}_6\text{H}_5 \end{array}$
$\approx +26.0$	+111.6	-81.2	-39.3	-47.5
$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{CO}_2\text{H} \\ \\ \text{C}_6\text{H}_5 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{CO}_2\text{H} \\ \\ \text{C}_6\text{H}_5 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots \text{CH}_2\text{CO}_2\text{H} \\ \\ \text{C}_6\text{H}_5 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_2\text{CO}_2\text{H} \\ \\ \text{C}_6\text{H}_5 \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_3\text{CO}_2\text{H} \\ \\ \text{C}_6\text{H}_5 \end{array}$
<i>dextro</i>	+140	-83.3	-6.6	-22.1

* For all these phenyl acids, the first absorption region (λ 2700 to λ 2500) is inactive.

In all cases the dispersion can be expressed by two terms of opposite sign, the first term originating apparently in the carboxyl group.

The change in sign of rotation on passing from $n_2 = 0$ to $n_2 = 1$ in these series is due to change in sign of the partial rotation of the first contribution. For example, ethylphenylacetic and propylphenylpropionic acids have opposite rotation but exhibit the same type of dispersion ($\lambda_0 \approx 2300 \text{ \AA}$ for a single-term formula). In the phenyl series having

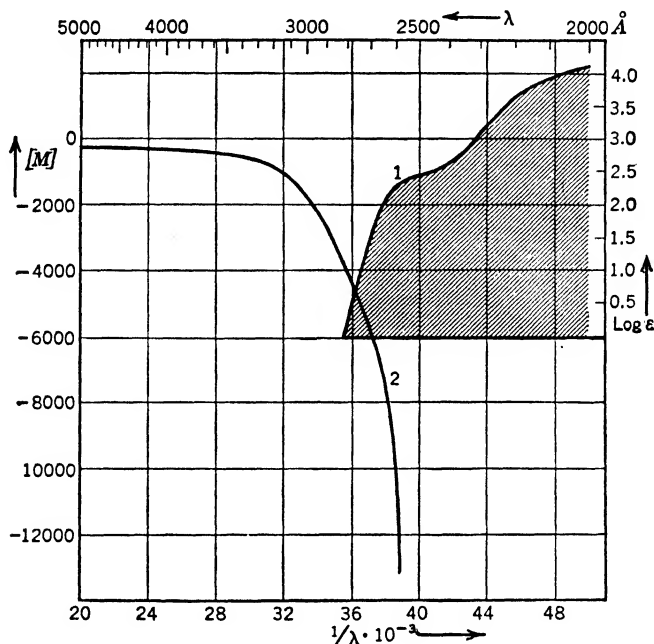


FIG. 10.*—Rotatory dispersion and absorption of *levo*-ethyl mandelate in alcohol (Kuhn and Biller).

$n_1 = 0$ and $n_3 = 2$, the rotatory dispersion was measured in four members; for the member having $n_2 = 0$, $\lambda_0 = 2100 \text{ \AA}$; $n_2 = 1$, $\lambda_0 = 2300 \text{ \AA}$; $n_2 = 2$, $\lambda_0 = 2400 \text{ \AA}$; and $n_2 = 3$, $\lambda_0 = 2600\text{--}2700 \text{ \AA}$ (from measurements in the visible region). In the last case the dispersion curve has a strong curvature and cannot be expressed even for the visible region by a single term. These values show that with progressive increase in n_2 there is a progressive increase in the dispersive power of the substances, the values of the two partial rotations coming nearer together.

The dispersion curves of 2-cyclohexylpropionic and 3-cyclohexylbutyric acids present *clear evidence* that here the rotation in the visible

* Kuhn and Biller, *Z. physik. Chem.*, B **29**, 1 (1935). (Courtesy of publishers.)

region is determined by the partial rotation of the carboxyl group. The dispersion constants for 2-cyclohexylpropionic acid are $\lambda_0 = 2170 \text{ \AA}$ for a one-term formula (valid in the visible region) and $\lambda_1 = 1900 \text{ \AA}$, $\lambda_2 = 1400 \text{ \AA}$ for a two-term formula (valid for visible and ultra-violet regions).

In contrast to the acids discussed above stands hydroxyphenylacetic (mandelic) acid.³¹ The dispersion and absorption curves of the ethyl ester of this acid may be seen in Fig. 10. The first absorption of the

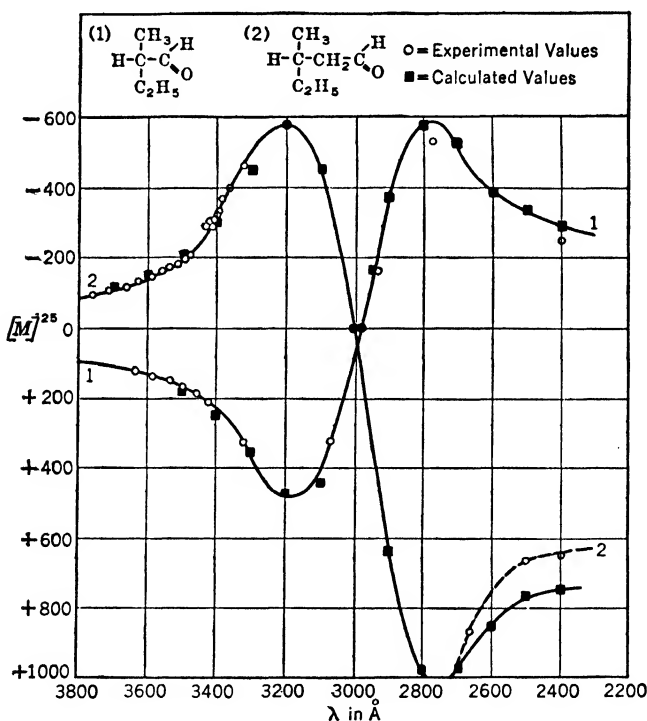


FIG. 11.*—Rotatory dispersion of two configurationally related aldehydes.

phenyl group is anisotropic and furnishes the major contribution to the rotation of the substance in the visible region. Thus the introduction of an hydroxyl in place of a methyl group in disubstituted acetic acid entirely changes the optical properties of the molecule.

Aldehydes. Analysis of this group of substances is simpler, owing to the position and the properties of the absorption bands of the aldehydic group. The first band at $\lambda 2950 \text{ \AA}$ is weak ($\epsilon \approx 20$), well isolated,

* Levene and Rothen, *J. Chem. Phys.*, **4**, 48 (1936). (Courtesy of publishers.)

and free from overlapping, thus permitting calculation of its partial rotation from the experimental dispersion curve within the region of the absorption band. Indeed, Lowry³⁵ used with advantage optically active aldehydes for the analysis of the course of dispersion within and without a given absorption band.

From Table X³⁶ it can be seen that the direction of rotation in the visible region changes on passing from $n_2 = 0$ to $n_2 = 1$. It can be seen from Fig. 11 that the change is actually due to the reversal of the partial rotation of the aldehydic group. In Fig. 12 are given the

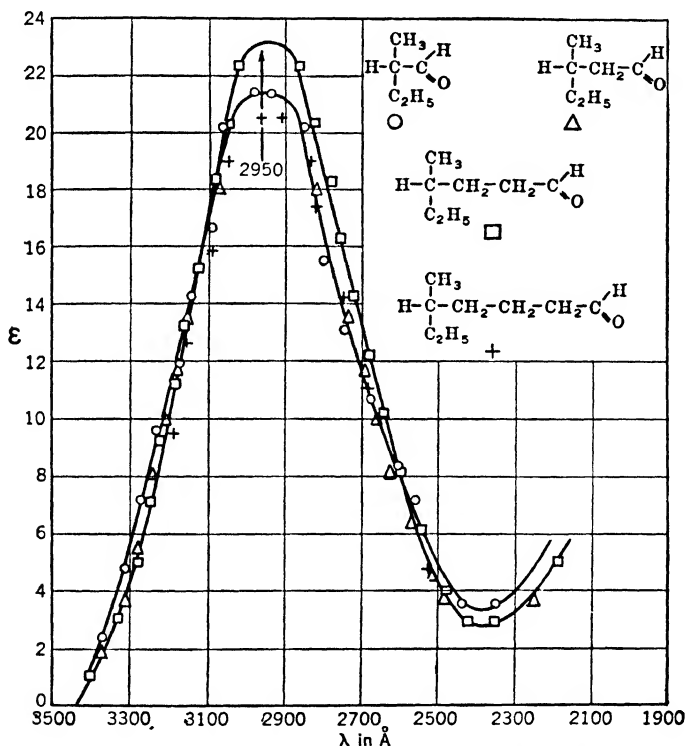


FIG. 12.*—Absorption curves of aliphatic aldehydes.

absorption curves of some aliphatic aldehydes; the curves are practically identical for the four members of the series, whereas the curves of the rotatory dispersion of the first two members are pretty nearly mirror images.

³⁵ Hudson, Wolfrom, and Lowry, *J. Chem. Soc.*, 1179 (1933).

³⁶ Levene and Rothen, *J. Chem. Phys.*, **4**, 48 (1936).

* Levene and Rothen, *J. Chem. Phys.*, **4**, 48 (1936). (Courtesy of publishers.)

TABLE X
CONFIGURATIONALLY RELATED ALIPHATIC ALDEHYDES
[M]_D²⁵ max. (in heptane)

$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{C} \begin{array}{l} \nearrow \text{O} \\ \searrow \text{H} \end{array} \\ \\ \text{C}_2\text{H}_5 \end{array}$ <p style="text-align: center;">+20.3</p>	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{CH}_2 - \text{C} \begin{array}{l} \nearrow \text{O} \\ \searrow \text{H} \end{array} \\ \\ \text{C}_2\text{H}_5 \end{array}$ <p style="text-align: center;">-8.7</p>	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_2 - \text{C} \begin{array}{l} \nearrow \text{O} \\ \searrow \text{H} \end{array} \\ \\ \text{C}_2\text{H}_5 \end{array}$ <p style="text-align: center;">+12.0</p>	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_3 - \text{C} \begin{array}{l} \nearrow \text{O} \\ \searrow \text{H} \end{array} \\ \\ \text{C}_2\text{H}_5 \end{array}$ <p style="text-align: center;">+12.8</p>
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Comparing the mathematical expression for the dispersion curves of the four substances, as given below (Table XI), it becomes evident that there is an unmistakable periodic change in the direction of the shifts of the partial rotation of the band λ 2950 Å with the progressive increase in the value of n_2 .

Conclusion. Comparing the direction of the partial rotation of the carboxyl group in aliphatic and phenyl aliphatic acids with the partial rotation of the carbonyl group in aldehydes it was found that they were of the same sign in members having the same value for n_2 .

TABLE XI

$$\begin{aligned} \text{2-Methylbutanal-1 } [M]_{\text{max.}}^{25} &= + \frac{5.104}{\lambda^2 - 0.932} \\ \text{3-Methylpentanal-1 } [M]_{\text{max.}}^{25} &= - \frac{7.569}{\lambda^2 - 0.093} + \frac{6.693}{\lambda^2 - 0.032} \\ \text{4-Methylhexanal-1 } [M]_{\text{max.}}^{25} &= - \frac{0.371}{\lambda^2 - 0.087} + \frac{4.114}{\lambda^2 - 0.036} \\ \text{5-Methylheptanal-1 } [M]_{\text{max.}}^{25} &= - \frac{0.8158}{\lambda^2 - 0.087} + \frac{4.9102}{\lambda^2 - 0.036} \end{aligned}$$

Second Category. The interest of organic chemistry in the analysis of the dispersion curves of substances of known configuration lay in the promise they offered to formulate a rational basis for correlating the configuration of substances for which classical organic chemistry did not offer an adequate method. It was therefore of special interest to compare the course of events in aliphatic azides and halides.

*Aliphatic Azides.*³⁰ The azido group, $-\text{N}_3$, shares many of the chemical properties of the halogens and may be regarded as a pseudo-halogen. On the other hand, azides are readily reduced to amines. Amines, in their turn, from the viewpoint of optical rotation, resemble alcohols. For alcohols and amines it is possible to establish the con-

figural relationship between members having $n_2 = 0$ and those having $n_2 > 1$ on the basis of adequate indirect methods. Hence it is possible to establish such relationships also for the azides.

It has been accepted by many workers in this field that, from the viewpoint of optical rotation, the $-N_3$ group behaves similarly to a halogen. If this were true, the correlation of the configurations of secondary halides with those of secondary alcohols would be self-evident. Therefore, a comparison of the behavior of azides with respect to progressive change in the value of n_2 becomes of special interest. In Table XII are shown the rotations of configurationally related azides.

Two absorption regions can be distinguished in the azido group, one weak band at λ 2880 Å and a strong absorption from λ 2200 Å down. Analysis of rotatory dispersion curves revealed the fact that, in the alkyl azides thus far examined, no Cotton effect could be detected in the band

TABLE XII
CONFIGURATIONALLY RELATED AZIDES
[M]_D²⁵ max. (in heptane)

	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{N}_3 \\ \\ \text{C}_2\text{H}_5 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{CH}_2\text{N}_3 \\ \\ \text{C}_2\text{H}_5 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{CH}_2\text{CH}_2\text{N}_3 \\ \\ \text{C}_2\text{H}_5 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{CH}_2\text{CH}_2\text{CH}_2\text{N}_3 \\ \\ \text{C}_2\text{H}_5 \end{array}$
[M] _D ²⁵ max.	+40.0	+10	+26	+17
[M] ²⁵ max.	$+\frac{12.712}{\lambda^2-0.0296}$	$+\frac{3.211}{\lambda^2-0.03625}$	$+\frac{8.300}{\lambda^2-0.0352}$	$+\frac{5.338}{\lambda^2-0.0314}$

nearest to the visible region, whereas the more distant absorption regions furnish the principal contribution to the rotation in the visible region. In the lower members of all series, independent of the value of n_2 , the dispersion curves are satisfactorily expressed by a one-term formula. (See Table XII.) The dispersion of the higher members of the series having $n_2 = 1$ and homologous with respect to n_3 are anomalous, their rotations in the visible region being of opposite direction to those of the lower members.

Halides. New information has been obtained in recent years on both absorption and rotatory dispersion of halides. Price³⁷ has shown that the absorption spectra of organic iodides, bromides, and chlorides are analogous. With decreasing wave lengths there is found a broad region of continuous absorption with a maximum located at λ 2600 Å for the iodides, λ 1900 Å for the bromides, and λ 1720 Å for the chlorides.

³⁷ Price, *ibid.*, 4, 539 (1936).

For smaller wave lengths, the absorption may be resolved into lines which fit two series of Rydberg. They converge toward two limits corresponding to the respective ionization potentials. Rotatory dispersion measurements have been made on a series of configurationally related halides. In Table XIII are given the rotations of bromides of

TABLE XIII
CONFIGURATIONALLY RELATED BROMIDES $[M]_D^{25}$ max. (homogeneous)

$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{CH}_2\text{Br} \\ \\ \text{C}_2\text{H}_5 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_2\text{Br} \\ \\ \text{C}_2\text{H}_5 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_3\text{Br} \\ \\ \text{C}_2\text{H}_5 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_4\text{Br} \\ \\ \text{C}_2\text{H}_5 \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_5\text{Br} \\ \\ \text{C}_2\text{H}_5 \end{array}$
+7.9	+38.8	+21.9	+14.9	+14.0
$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots \text{CH}_2\text{Br} \\ \\ \text{C}_2\text{H}_7(n) \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_2\text{Br} \\ \\ \text{C}_2\text{H}_7(n) \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_3\text{Br} \\ \\ \text{C}_2\text{H}_7(n) \end{array}$	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H} \cdots \text{C} \cdots (\text{CH}_2)_4\text{Br} \\ \\ \text{C}_2\text{H}_7(n) \end{array}$	
<i>low dextro</i>	+21.0	+14.5	+7.8	

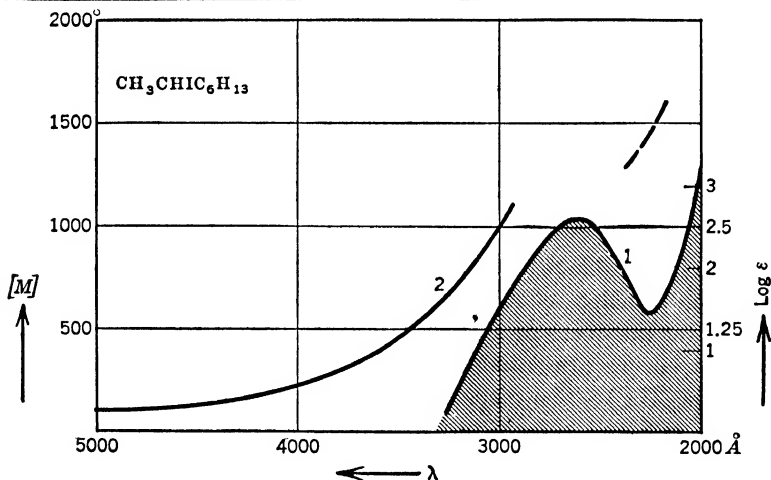


FIG. 13.*—Rotatory dispersion and absorption of *dextro*-2-iodooctane in hexane (Kuhn and Biller).

only two series homologous with respect to n_2 . Several other series, not only of bromides but also of iodides and chlorides, were analyzed, inasmuch as each halide has its advantages and disadvantages for the study of the dispersive power of halides as a class. A periodicity in the

* Kuhn and Biller, *Z. physik. Chem.*, **B 29**, 256 (1935). (Courtesy of publishers.)

shift of rotation similar to that in the corresponding azides is observed in the members having $n_2 = 1, 2$, and 3 . The members having $n_2 = 0$ are not given in Table XIII because their configuration cannot be established by methods of classical organic chemistry and hence their configurational relationship will be a matter for separate discussion.

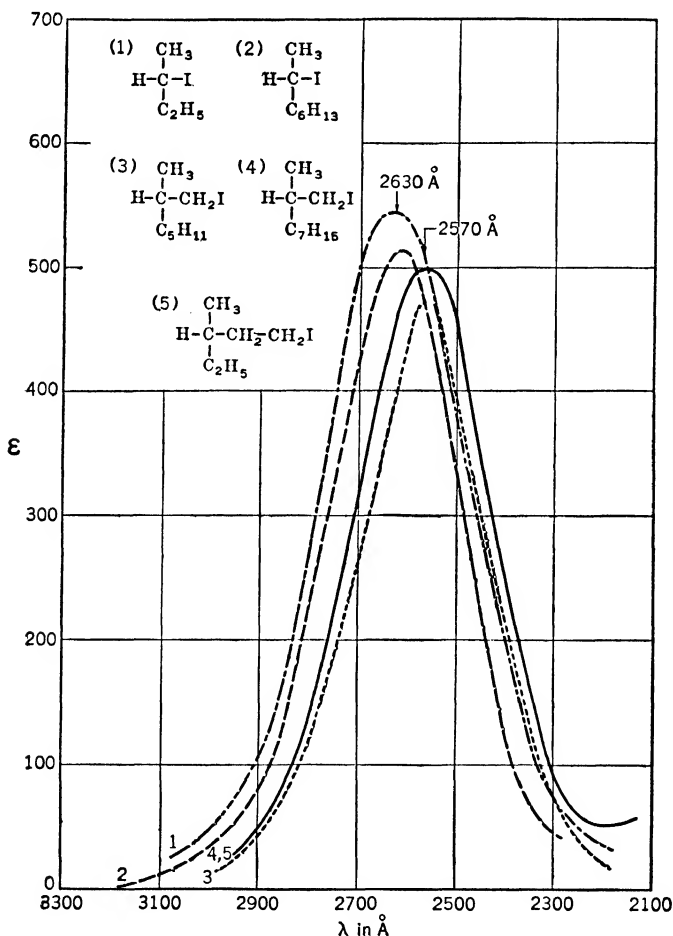


FIG. 14.*—Absorption spectra of iodides.

Measurements of the rotatory dispersion of secondary iodides have been reported from two laboratories. Kuhn and Biller³⁸ gave the dispersion curve of 2-iodooctane in heptane showing that the first absorp-

* Levene, Rothen, and Marker, *J. Chem. Phys.*, **4**, 442 (1936). (Courtesy of publishers.)

³⁸ Kuhn and Biller, *Z. physik. Chem.*, **B 29**, 256 (1935).

tion band of the iodine furnishes only a very small part of the rotation in the visible region (Fig. 13). No detailed record of the measurements is given in the paper.

On the other hand, Levene and Rothen's³⁹ results are consistent with the view that the partial rotation of the first absorption region adds materially to the rotation of the substances in the visible region.

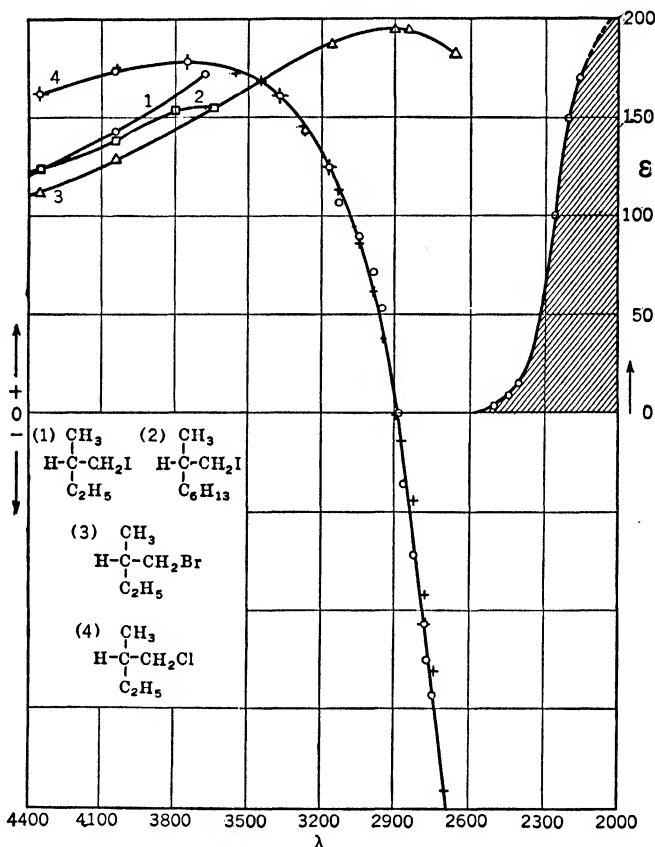


FIG. 15.*—Rotatory dispersion curves of primary halides. The ordinate represents the rotations in arbitrary units. The absorption curve of the bromide may be seen to the right (shaded surface).

Absorption curves of primary and secondary iodides are given in Fig. 14, and rotatory dispersions of several iodides, bromides, and chlorides may be seen in Fig. 15.

³⁹ Levene, Rothen, and Marker, *J. Chem. Phys.*, **4**, 442 (1936).

* Levene, Rothen, and Marker, *J. Chem. Phys.*, **4**, 442 (1936). (Courtesy of publishers.)

TABLE XIV

EQUATIONS REPRESENTING THE MAXIMUM MOLECULAR ROTATIONS OF THE IODIDES, BROMIDES, AND CHLORIDES (IN HOMOGENEOUS STATE) VALID IN THE VISIBLE AND THE NEAR-ULTRA-VIOLET SPECTRUM

<i>Levo</i> -2-Iodobutane	$[M]_{\text{max}}^{25} = -\frac{9.8023}{\lambda^2 - 0.0685} - \frac{7.2490}{\lambda^2 - 0.040}$		
<i>Levo</i> -2-Iodoöctane	$[M]_{\text{max}}^{25} = -\frac{13.312}{\lambda^2 - 0.069} - \frac{15.162}{\lambda^2 - 0.040}$		
	$[M]_{\text{max}}^{25} = -\frac{12.226}{\lambda^2 - 0.069} - \frac{18.175}{\lambda^2 - 0.040}$		
	(in heptane)		
<i>Dextro</i> -1-Iodo-2-Methylbutane	$[M]_{\text{max}}^{25} = -\frac{4.7539}{\lambda^2 - 0.043} + \frac{8.5535}{\lambda^2 - 0.028}$		
<i>Dextro</i> -1-Iodo-3-Methylpentane	$[M]_{\text{max}}^{25} = +\frac{14.133}{\lambda^2 - 0.0466}$		
<i>Dextro</i> -1-Iodo-4-Methylhexane	$[M]_{\text{max}}^{25} = +\frac{8.377}{\lambda^2 - 0.0336}$		
<i>Levo</i> -2-Bromobutane	$[M]_{\text{max}}^{25} = -\frac{12.614}{\lambda^2 - 0.032} + \frac{3.482}{\lambda^2}$		
<i>Levo</i> -2-Bromoöctane	$[M]_{\text{max}}^{25} = -\frac{22.159}{\lambda^2 - 0.034} - \frac{2.540}{\lambda^2 - 0.025}$		
	$+ \frac{5.434}{\lambda^2 - 0.010}$		
<i>Dextro</i> -1-Bromo-2-Methylbutane	$[M]_{\text{max}}^{25} = -\frac{9.4151}{\lambda^2 - 0.032} + \frac{12.169}{\lambda^2 - 0.025}$		
<i>Dextro</i> -1-Bromo-3-Methylpentane	$[M]_{\text{max}}^{25} = +\frac{12.243}{\lambda^2 - 0.0317}$		
<i>Levo</i> -2-Chlorooctane	$[M]_{\text{max}}^{25} = -\frac{15.966}{\lambda^2 - 0.024} - \frac{7.329}{\lambda^2 - 0.020}$		
	$+ \frac{10.820}{\lambda^2 - 0.012}$		
<i>Dextro</i> -1-Chloro-2-Methylbutane	$[M]_{\text{max}}^{25} = -\frac{3.667}{\lambda^2 - 0.030} + \frac{4.352}{\lambda^2 - 0.020}$		
<i>Dextro</i> -1-Chloro-3-Methylheptane	$[M]_{\text{max}}^{25} = +\frac{4.6373}{\lambda^2 - 0.025} - \frac{2.1879}{\lambda^2 - 0.015}$		
<i>Dextro</i> -1-Chloro-5-Methylnonane	$[M]_{\text{max}}^{25} = +\frac{1.763}{\lambda^2 - 0.025} - \frac{1.059}{\lambda^2 - 0.015}$		

The mathematical expressions for the maximum rotations of several secondary and primary halides are given in Table XIV. In Table XV, their relative partial rotations are compared with the relative partial rotations of a series of azides which are configurationally related to the halides, the members having $n_2 = 0$ excepted.

TABLE XV

DIRECTIONS AND RELATIVE VALUES OF THE PARTIAL CONTRIBUTIONS OF THE HALOGEN ATOM AND N_3 GROUP IN CONFIGURATIONALLY RELATED HALIDES AND AZIDES

	n_2	0	1	2	3
Halides	First band	Moderate	0	($\simeq 0$)	0
	Second region	Strong	—	++	0
	Third region	Weak	++	—	++
	Fourth (in the main in rest of the molecule)		?	?	—
Azides	First band	0	0	0	0
	Second region	?	++	++	++
	Third region	+	—	—	—

The relative strength of rotation is indicated by the number of (+) or (—) signs.

The configurational relationship of azides and halides having $n_2 = 0$ is not known, and the question is: Can they be correlated on the basis of the optical relationship of the primary azides to the primary halides? Comparing the configurationally related members it is seen that, in those having $n_2 = 2$ or 3, the nearest optically active absorption region is positive and the more distant negative in the azides as well as in the halides. When $n_2 = 1$, all halides homologous with respect to n_3 have the same sign of rotation and their dispersion curves are anomalous. On the other hand, in the azides having $n_2 = 1$ the dispersion curves are normal for the lower homologs with respect to n_3 and anomalous for the higher members. The sign of rotation of the higher members is opposite to that of the lower ones as well as that of the corresponding halides. *Thus, the assumption that configurationally related azides and halides always rotate in the same direction is no longer tenable.* The optical behavior of the azides, configurationally related to the corresponding halides, offers no clue to the correlation of the configurations of secondary halides with secondary azides.

CORRELATION OF CONFIGURATIONS: α -SUBSTITUTED CARBOXYLIC ACIDS
(VICINAL EFFECT OF HALOGENO AND OF AZIDO GROUPS)

Carboxylic acids were chosen by Kuhn in 1930 for the application of his theoretical considerations to the analysis of rotatory dispersion curves and for the correlation of the configurations of substances when classical organic chemistry offers no adequate method. The α -halogeno and α -azido acids offer the advantage that their first absorption regions are anisotropic, whereas in the alkyl and aryl derivatives they are either entirely inactive or they furnish only a small partial rotation. Substances with several absorption bands located at close intervals in the spectrum present difficulties in analysis because of the overlapping of several partial rotations.

Of all the α -substituted carboxylic acids, the azido and mercapto acids present advantages over the halogeno acids since they permit significant chemical changes in the carboxyl as well as in the azido or in the mercapto group without Walden inversion.

Azido Acids. In Fig. 16 are given the total rotations and absorption curve as well as the partial rotations of the N_3 group and of the rest of the molecule in the *dextro* methylester of azidopropionic acid measured by Kuhn and Braun. From these curves it can be seen that the maximum of the partial rotation of the azido group is located at $\lambda \simeq 3100 \text{ \AA}$ and that the greatest part of the rotation in the visible region is furnished by what seems to be the partial contribution of the CO_2CH_3 group.

Very similar were the results of the measurements of the rotatory dispersion of the free *dextro* acid and of its *levo* sodium salt obtained by Levene and Rothen²³ who found

$$[M]^{25} = -\frac{2.526}{\lambda^2 - 0.080} + \frac{4.710}{\lambda^2 - 0.020}$$

for the acid. The salt has a normal dispersion which can be expressed for the visible region by a one-term formula with $\lambda_0 = 3160 \text{ \AA}$.

Thus in the *dextro* free acid and its ester, the first partial rotation is negative whereas the second is positive and predominates, the dispersion in the visible region being anomalous. On the other hand, in the dimethylamide of the same acid, both partial rotations are negative, as can be seen from Fig. 17. Again, the second partial rotation furnishes the greater contribution in the visible region.

It is of interest to inquire into the effect produced on the partial rotation of the carboxyl group by the reduction of the azido to an amino

group, and also to compare the direction of the partial rotation of the amino group with that of the azido group. Dextrorotatory azido-propionic acid leads on reduction to levorotatory alanine, which exhibits a normal dispersion.²³ Unfortunately, the absorption bands of the two

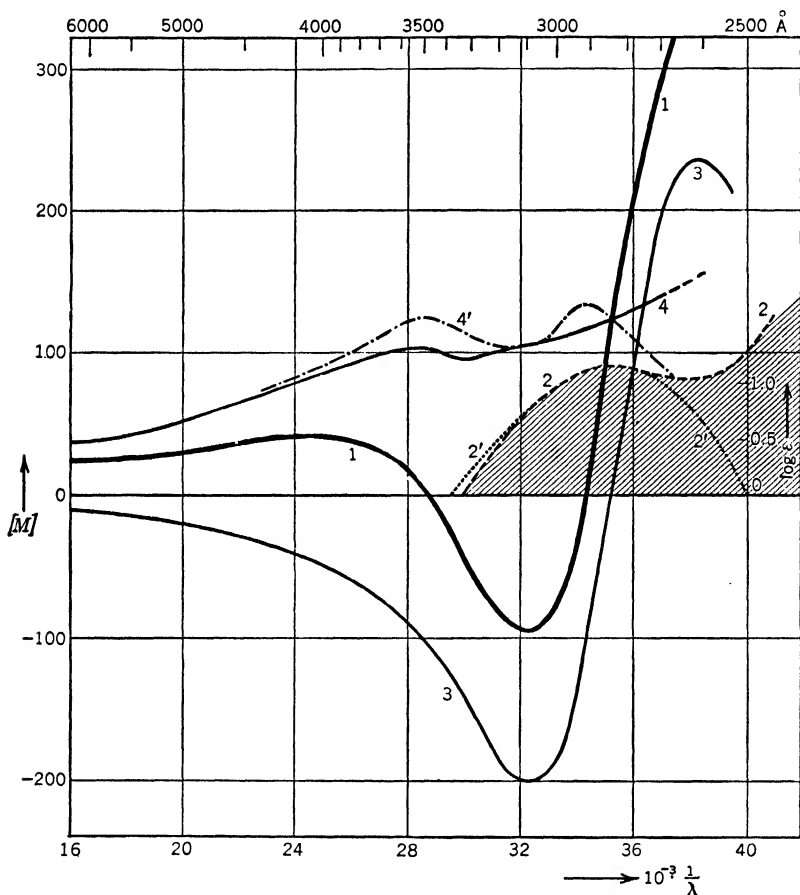


FIG. 16.*—*Dextro* methyl ester of azidopropionic acid. 1. Rotatory dispersion in hexane. 2. Absorption in hexane. 3. Partial rotation of λ_{N_2} 2900 Å. 4. Difference between curves 1 and 3 (Kuhn and Braun).

chromophoric groups overlap so much that the origin of the first partial rotation is very difficult to determine. However, there is sufficient reason to believe that the partial rotation of the carboxyl group is levorotatory, since the levorotation of the hydrochloride of the amino acid is

* Kuhn and Braun, *Z. physik. Chem.*, B 8, 281 (1930). (Courtesy of publishers.)

greater than that of its sodium salt. Thus the sign of the partial rotation of the carboxyl is different in the configurationally related amino and azido acids. The direction of the rotatory contribution of $\text{CON}(\text{CH}_3)_2$ is opposite to that of the CO_2CH_3 group in the azido acids.

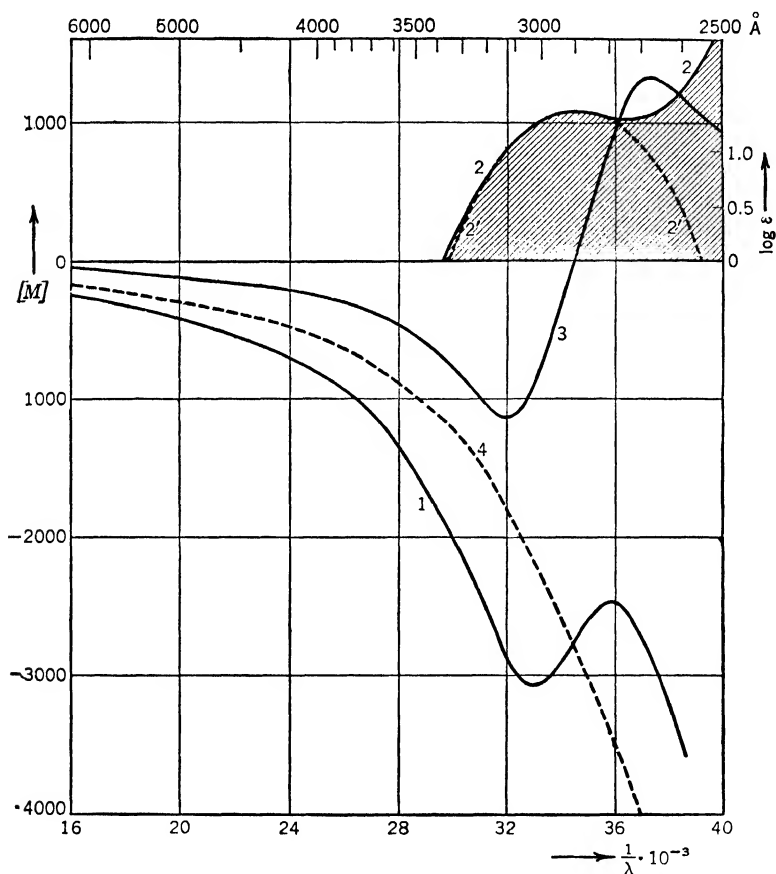


FIG. 17.*—*Levo* dimethylamide of azidopropionic acid. 1. Rotatory dispersion in ether. 2. Absorption in ether. 3. Partial rotation of λ_{N_D} 2900 Å. 4. Difference between curves 1 and 3 (Kuhn and Braun).

Mercapto and Sulfonic Acids.¹⁶ In some respects the mercapto and the corresponding sulfonic acids are more satisfactory material than the azido and the corresponding amino acids. The advantage of these series of acids lies in the fact that the sulfonic acids present less doubt as to the origin of the first partial rotation than do the amino acids.

* Kuhn, *Trans. Faraday Soc.*, **26**, 293 (1930); Kuhn and Braun, *Z. physik. Chem.*, **B 8**, 281 (1930). (Courtesy of publishers.)

The absorption curves of the two mercapto acids studied exhibit a definite maximum at λ 2400 Å. This band can safely be attributed to the SH group, since 2-mercaptobutane has in the same part of the spectrum an absorption band, which, however, is slightly displaced toward higher frequencies (as is to be expected), and yet is still in a region of transparency for a simple aliphatic acid. The absorption curves of the

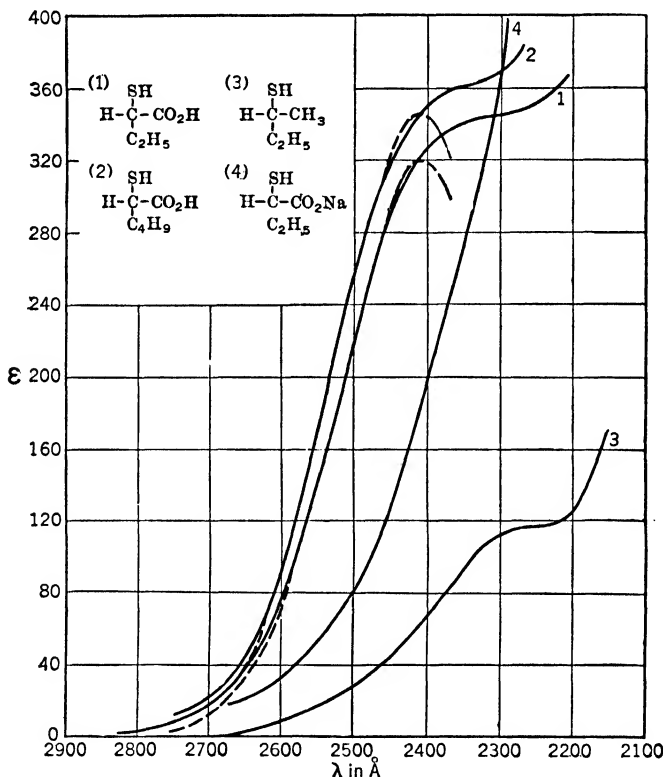


Fig. 18.*—Absorption curves of the mercapto acids and their sodium salts. The dotted curve is calculated.

sodium salts are less characteristic owing to the appearance of a strong second band (COO^-) which overlaps the SH band and masks its maximum (see Fig. 18).

The absorption curves of the sulfonic acids present a characteristic maximum at λ 2150 Å (see Fig. 19). This band is doubtless the first CO_2H band displaced toward lower frequencies by the proximity of CO_2H to SO_3H , since an ordinary alkylsulfonic acid (2-methylpropylsulfonic

* Levene and Rothen, *J. Chem. Phys.*, **2**, 681 (1934). (Courtesy of publishers.)

acid (1)) has practically no absorption at all in that region and absorbs much less than the corresponding aliphatic acid. The absorption curves of the sodium salts present a striking difference. The curve is displaced toward higher frequencies by about 150 \AA , the maximum of the first band can no longer be seen, and a continuous increasing absorption is observed as the wave length becomes smaller. The fact that two

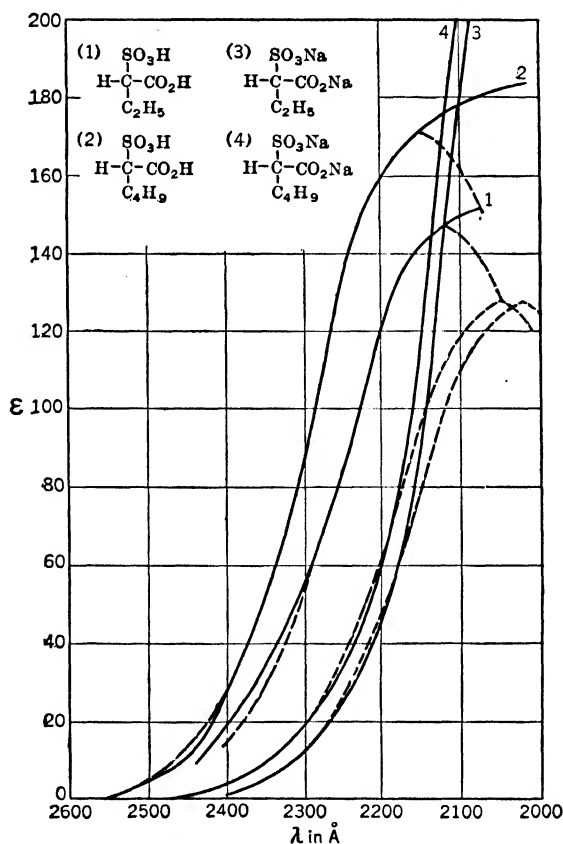


Fig. 19.*—Absorption curves of sulfonic acids and their sodium salts. The dotted curve is calculated.

bands overlapping each other are present and not one broad band is very well demonstrated by analysis of both absorption and dispersion curves. The striking change in the absorption is brought about by the ionization of the CO_2H group since the SO_3H group is already completely ionized in the free acid, as electrometric titration curves have shown.

* Levene and Rothen, *J. Chem. Phys.*, **2**, 681 (1934). (Courtesy of publishers.)

The analysis of the rotatory dispersion of the two mercapto acids led to the following values of their rotation:

$$\text{Dextro-}\alpha\text{-mercaptobutyric acid } [M]^{25} = \frac{25.495}{\lambda^2 - 0.060} - \frac{19.578}{\lambda^2 - 0.043} \quad \left. \begin{array}{l} \lambda_1 = 2450 \text{ \AA} \\ \lambda_2 = 2070 \text{ \AA} \end{array} \right\}$$

$$\text{Dextro-}\alpha\text{-mercaptocaproic acid } [M]^{25} = \frac{28.415}{\lambda^2 - 0.060} - \frac{20.140}{\lambda^2 - 0.045} \quad \left. \begin{array}{l} \lambda_1 = 2450 \text{ \AA} \\ \lambda_2 = 2120 \text{ \AA} \end{array} \right\}$$

The two respective partial rotations do not change sign in the mono- and the disodium salt.

The measurements of the rotatory dispersion of the two sulfonic acids corresponding to the two thio acids led to the following expressions:

$$(1) \text{ Dextro-}\alpha\text{-sulfobutyric acid } [M]^{25} = \frac{3.0656}{\lambda^2 - 0.045} - \frac{2.1733}{\lambda^2 - 0.030}$$

$$\text{Levo-disodium salt } [M]^{25} = -\frac{36.0395}{\lambda^2 - 0.041} + \frac{28.640}{\lambda^2 - 0.037}$$

$$(2) \text{ Levo-}\alpha\text{-sulfocaproic acid } [M]^{25} = \frac{3.0626}{\lambda^2 - 0.0462} - \frac{6.5309}{\lambda^2 - 0.007}$$

$$\text{Levo-disodium salt } [M]^{25} = -\frac{37.437}{\lambda^2 - 0.042} + \frac{21.271}{\lambda^2 - 0.037}$$

Thus on passing from the mercapto acids to the sulfonic acids the partial rotations of the carboxyl groups change sign just as on passing from azido to amino acids.

On the other hand, in the disodium salts of the sulfonic acids the conditions are reversed and the partial rotations of the carboxyl groups have the sign which they had in the thio acids.

α -Halogeno Acids. It was shown in the section on alkyl halides and azides that the configuration of these substances cannot be correlated on the basis of the direction of the partial rotation of the halogen atom and of the azido group, inasmuch as in substances of known configuration, depending on their structure, the halogen atom furnished a partial rotation of the same sign as, or of opposite sign from, that of the azido group. It was important to see whether α -halogeno- and α -azidocarboxylic acids would present less difficulty in the correlation of their configurations on the basis of the analysis of their rotatory dispersions, for it is possible to correlate the configurations of the alkyl halides with those of the α -halogeno acids and of alkyl azides with the corresponding α -azidocarboxylic acids. Hence, the solving of one problem would simultaneously solve the others.

α -Iodopropionic Acid. The free acid, its salt, the ethyl ester, and dimethylamide have been analyzed. In Fig. 20 are shown the absorption curves of iodopropionic acid and of its sodium salt. The dispersion curve of the dextrorotatory acid can be represented by the expression:

$$[M]^{25} = \frac{26.379}{\lambda^2 - 0.0806} - \frac{13.687}{\lambda^2 - 0.042}$$

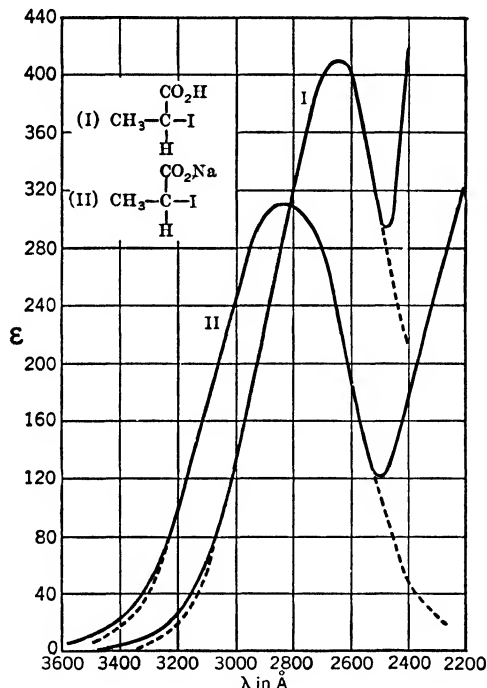


Fig. 20.*—Absorption curves of α -iodopropionic acid and its sodium salt.

This equation indicates that in the dextrorotatory acid the long-wave-length absorption band of the iodine atom determines the rotation in the visible region and that the partial rotation of the carboxyl group is levorotatory.

The dispersion curve of the salt is anomalous, the negative partial rotation (probably of the carboxyl) being dominant in the visible region.

Fig. 21 shows the absorption and dispersion of the configurationally related *dextro* ester and *levo* dimethylamide.³⁸ It may be concluded that in the ester the rotation in the visible region is determined princi-

* Levene and Rothen, *J. Biol. Chem.*, **107**, 533 (1934). (Courtesy of publishers.)

pally by the iodine atom, even though it seems that the first absorption region of the iodine atom is only slightly anisotropic. The dispersion curve of the dimethylamide is anomalous.

From this it follows that in all derivatives of *dextro*-iodopropionic acid the partial contribution of the iodine atom remains dextrorotatory.

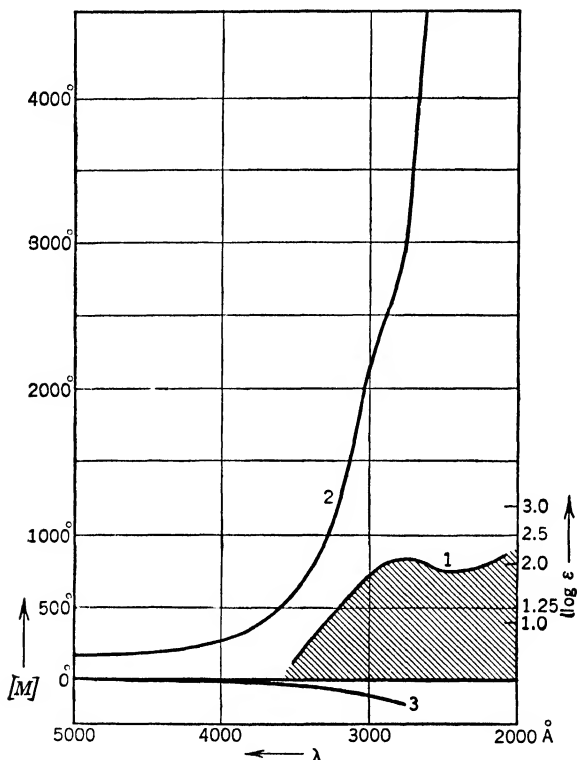


FIG. 21.*—(1) Absorption and (2) dispersion of *dextro* methyl ester of iodopropionic acid in hexane. (3) Dispersion of *levo* dimethylamide of iodopropionic acid in hexane (Kuhn and Biller).

Thus the conditions in the *dextro*-azido- and in the *dextro*-iodopropionic acids are similar in one respect, namely, in both, the direction of the partial rotation of the first absorption band remains unaffected by chemical changes in the carboxyl group. They differ, however, with respect to the direction of the partial rotation of the first absorption band, which is levorotatory in the azido group, and dextrorotatory in the halogeno acids.

* Kuhn and Biller, *Z. physik. Chem.*, **B 29**, 256 (1935). (Courtesy of publishers.)

α -Bromocarboxylic Acids. The absorption and dispersion curves of the ethyl ester of α -bromopropionic acid made by Kuhn are given in Fig. 22. From these curves it can be seen that the direction of rotation of the ester in the visible region is determined by the partial rotation of the bromine atom and that the rotatory contribution of the $-\text{CO}_2\text{C}_2\text{H}_5$

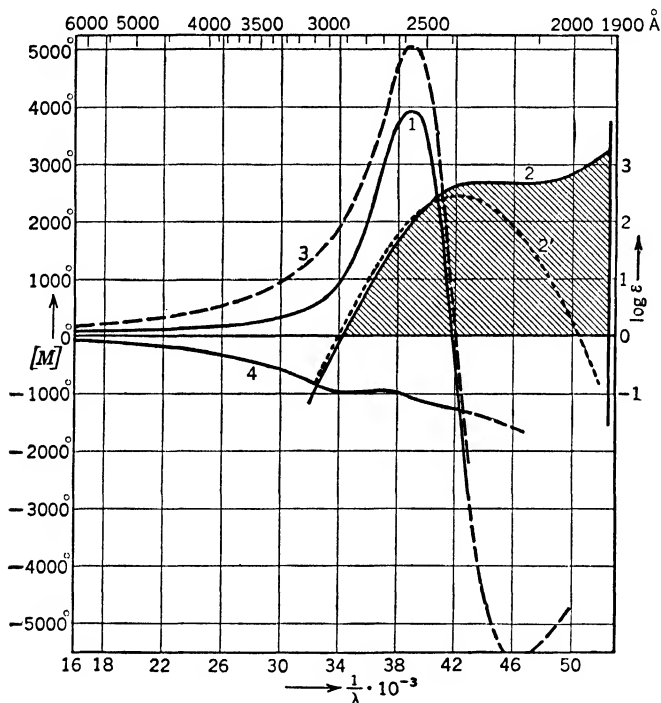


FIG. 22.*—*Dextro* ethyl ester of bromopropionic acid. 1. Rotatory dispersion in hexane. 2. Absorption in hexane. 3. Partial rotation of bromine. 4. Difference between curves 1 and 3 (Kuhn and Braun).

group is of opposite sign to that of the halogen, the conditions resembling those prevailing in the ester of the corresponding iodo acid.

The dispersion of α -bromopropionic acid⁴⁰ in heptane can be expressed by the equation

$$[M]^{25} = \frac{37.3417}{\lambda^2 - 0.053} - \frac{28.544}{\lambda^2 - 0.042} \text{ for } \lambda > 0.3190$$

$$\therefore \lambda_1 = 2300 \text{ \AA} \quad \text{and} \quad \lambda_2 = 0.2050 \text{ \AA}$$

* Kuhn and Braun, *Z. physik. Chem.*, **B** 8, 281 (1930). (Courtesy of publishers.)

⁴⁰ Levene and Rothen, *J. Biol. Chem.*, **107**, 533 (1934).

and that of α -bromocaproic acid by the equation:

$$[M]^{25} = \frac{16.416}{\lambda^2 - 0.0547} - \frac{1.622}{\lambda^2 - 0.042} \therefore \lambda_1 = 2340 \text{ \AA} \quad \text{and} \quad \lambda_2 = 2000 \text{ \AA}$$

The absorption curves of these two free acids and of their salts are given in Fig. 23.

The sodium salt of α -bromopropionic acid has a rotation opposite to that of the free acid and also to that of the salt of α -bromocaproic

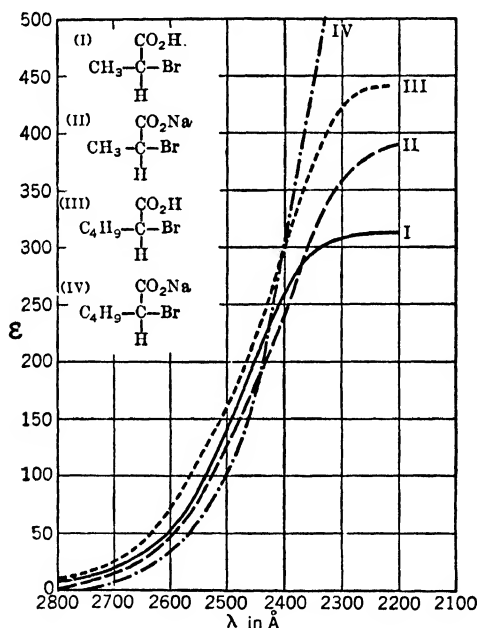


FIG. 23.*—Absorption curves of α -bromopropionic and α -bromocaproic acids and their sodium salts.

acid. The dispersion curve of the salt of the lower homolog is normal and that of the salt of the higher homolog is anomalous. Hence, the direction of rotation of the first two partial rotations changes sign in passing from the acids to the salts. In that respect the bromo acids are different from the iodo acids.

α -Chloropropionic Acid. The chloro derivatives offer advantages over the other halogeno derivatives by their greater transparency, which permits measurements of optical rotation to be extended into the more

* Levene and Rothen, *J. Biol. Chem.*, **107**, 533 (1934). (Courtesy of publishers.)

distant ultra-violet region of the spectrum. Figs. 24 and 25 represent the record of absorption and rotatory dispersion measurements made by Kuhn and Wolf on the *dextro* methyl ester and the configurationally related *levo* dimethylamide of α -chloropropionic acid.

The partial rotation of the chlorine atom is dextrorotatory, and the partial rotation of the $\text{CO}_2\text{C}_2\text{H}_5$ group and of the $\text{CON}(\text{CH}_3)_2$ group

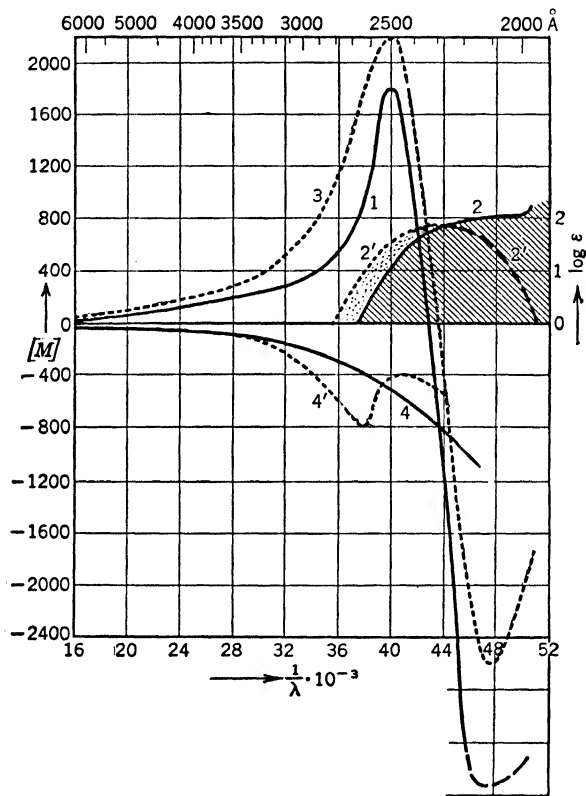


FIG. 24.*—*Dextro* methyl ester of chloropropionic acid. 1. Rotatory dispersion in hexane. 2. Absorption in hexane. 3. Partial rotation of chlorine. 4. Difference between curves 1 and 3 (Kuhn, Freudenberg, and Wolf).

is negative in both cases. The difference in the direction of rotation in the visible region is due entirely to the relative values of the partial rotations of the $\text{CO}_2\text{C}_2\text{H}_5$ and of the $\text{CON}(\text{CH}_3)_2$ group.

In Table XVI are summarized qualitatively the data obtained from the analysis of the substances discussed in this section. Before analyz-

* Kuhn, Freudenberg, and Wolf, *Ber.*, **63**, 2367 (1930). (Courtesy of publishers.)

ing the table it should be emphasized that in this group of substances the weak bands of the substituents are anisotropic, whereas, as was seen earlier, these bands in the alkyl derivatives are generally either not coupled at all or coupled feebly. The table reveals one property common to all the substances, namely, the partial rotation of the chromophoric group in the region of the longest wave length remains constant regardless of the changes introduced in the carboxyl group. *There are*

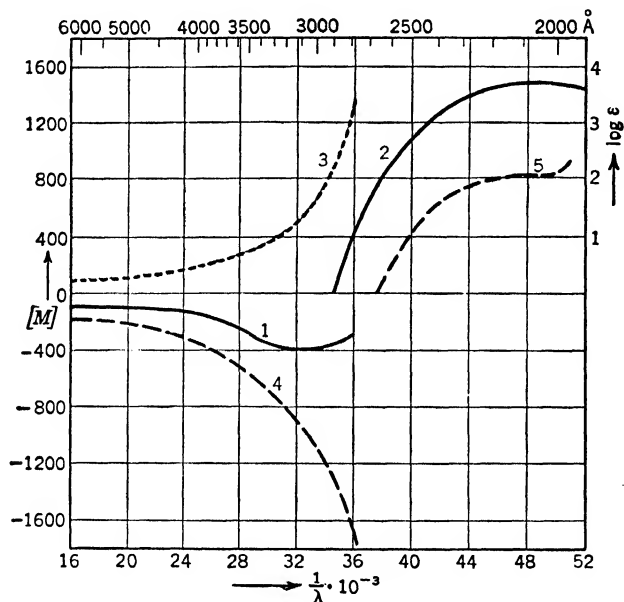


FIG. 25.*—*Levo* dimethylamide of chloropropionic acid. 1. Rotatory dispersion in hexane. 2. Absorption in hexane. 3. Partial rotation of chlorine. 4. Difference between curves 1 and 3 (Kuhn, Freudenberg, and Wolf).

two exceptions: the α -bromo- and α -sulfonic acids in which both partial contributions change sign on passing from the acids to the salts. The direction of rotation of the second partial rotation likewise remains constant in the majority of substances with the exception of the dimethylamides of the azido acids.

All the halogeno acids are configurationally related. The configurational relationship of the azido and of the thio acids to the halogeno acids cannot be correlated by methods of classical organic chemistry. It is known that the azido group chemically and electrochemically resembles a halogen atom and may be regarded as a pseudohalogen. It has

* Kuhn, Freudenberg, and Wolf, *Ber.*, **63**, 2367 (1930). (Courtesy of publishers.)

TABLE XVI
SUMMARY OF THE DIRECTION OF THE PARTIAL ROTATIONS IN THE SUBSTITUTED DEXTROROTATORY CARBOXYLIC ACIDS

	Azido Acid	Salt	CO ₂ C ₂ H ₅	CON(CH ₃) ₂	Amino Acid	Salt	Iodo Acid	Salt	CO ₂ C ₂ H ₅	CON(CH ₃) ₂	Bromo Acid	Salt	CO ₂ C ₂ H ₅	Chloro Acid	CO ₂ C ₂ H ₅	CON(CH ₃) ₂	Merapto Acid	Salt	Sulfonic Acid	Salt	
Rotation in visible.....	+	-	-	-	-	-	+	-	+	+	+	*	+	****	+	+	+	+	***	-	
Dispersion.....	An.	N	N	N	N	An.	N	N	N	N	N	N	N	N	N	N	N	N	An.	N	N
First absorption band.....	-	-	-	-	-	-	+	?	+	+	+	+	+	+	+	+	+	+	+	+	-
Second absorption band....	CO ₂ H	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

* Higher members dextrorotatory, anomalous.

** Higher members dextrorotatory, normal.

*** Higher members levorotatory, anomalous

**** No measurements were made on the free acid. Direction of partial rotations assumed by analogy with the ethyl ester.

therefore been accepted that configurationally related halides and azides rotate in the same direction. Is this assumption well founded?

Among the configurationally related alkyl halides and azides, as has been seen, are found some substances for which this assumption holds and others for which it is not valid. Thus, the assumption that α -azido-carboxylic and α -halogenocarboxylic acids rotate in the same direction may be right or wrong. To furnish irrefutable evidence based on rotatory dispersion measurements in favor of or against this assumption is for the present impossible. This statement sounds rather discouraging. It must be borne in mind, however, that our knowledge of the structure of the absorption bands in organic molecules is still imperfect. As yet, measurements of rotatory dispersion are seldom made beyond the first absorption region, and the conclusion as to the origin of the second major rotatory contribution is often uncertain because of the overlapping of absorption bands originating in different parts of the molecule. Future knowledge of the correlation of chemical structure and optical activity depends on further improvement in the methods of measurement and analysis of rotatory dispersion and on the elaboration of a more detailed theory of optical activity.

INFLUENCE OF EXTERNAL CONDITIONS

Temperature, Solvent, and Concentration. The influences of solvent, temperature, and concentration on optical activity and rotatory dispersion have been observed ever since Biot's early investigations, and the mechanism of these phenomena has been the subject of much speculation. Most of the earlier observations were made on substances of complex nature which rendered the analysis of the phenomena very difficult. In more recent years the effect of external factors on optical rotation was studied on substances having one asymmetric carbon atom. The credit for the pioneer work in this direction belongs to Pickard and Kenyon.

The earlier theories explained the effect of solvent on the basis of the molecular association either of the solute or of the solute and solvent. These theories were sponsored also in recent years by Rule, who emphasized the importance of the degree of polarity of solvent and solute. It may be said that these effects are more pronounced on substances where dispersions are expressed by two Drude terms of opposite sign. This phenomenon is easily understood if it is considered that solvent, temperature, and concentration affect each active band, that is, each Drude term separately—the terms with a high value for λ_i being undoubtedly more susceptible than the terms with low value for λ_i . Obviously the effect will be maximum when the observed rotation results from a difference of

two terms of nearly equal magnitude. An inversion of sign can then be expected since a small variation in the absolute values of the coefficients A_1 and A_2 is sufficient to produce the change from

$$\frac{A_1}{\lambda^2 - \lambda_1^2} > \frac{A_2}{\lambda^2 - \lambda_2^2} \quad \text{to} \quad \frac{A_1}{\lambda^2 - \lambda_1^2} < \frac{A_2}{\lambda^2 - \lambda_2^2}$$

A striking example of this has been reported by Kuhn, Freudenberg, and Seidler.⁴¹ Methoxyphenylethyl acetate has a rotation of $[M]_{5780}^{18} = -25^\circ$ in homogeneous state (anomalous dispersion) and $[M]_{5780}^{18} = +250^\circ$ in hexane (normal dispersion). This phenomenon can be observed in even very simple substances such as octanol-4 (propylbutylcarbinol), as was stated above, whose sign of rotation is not the same in homogeneous state and in ether; the sign of the partial contributions, however, remain unchanged.

It was natural to try to connect the effect of solvent with the shift observed in some absorption bands. The majority of changes in rotation produced by a solvent are brought about by the effect of the solvent on the absorption bands located in the long-wave-length region of the spectrum. However, according to the theories of Born and Kuhn, a very small variation in the distance or in the orientation of the coupled vibrations could produce an important effect on rotation and dispersion even without appreciable change in absorption. It is safe to say that the change in direction of rotation observed with change of temperature is caused by a similar phenomenon.

Index of Refraction. The influence of the index of refraction of the medium on the rotation was postulated by Born in his early theory. His calculations, corrected by Gans, led to the following relation: $\alpha = K(n^2 + 2)$. This formula, which expresses the non-specific effect of a solvent, was tested by Volkmann.⁴² He found it valid for a few substances and only when the active molecule had no electric moment. If one considers how frequently a slight change of n produces a considerable effect on rotation and dispersion of simple molecules, one must conclude that the specific effect is much greater than the non-specific, and that the formula given above is of little value.⁴³

Ionization and Salt Action. The fact that undissociated organic molecules and their ions have their own rotations has been known for a long time and has been emphasized by Landolt and by Oudemans. In recent years polarimetry has been used for measuring electrolytic dissociation.^{44, 45} That ionization should affect optical rotation is to be

⁴¹ Kuhn, Freudenberg, and Seidler, *Z. physik. Chem.*, **B 13**, 379 (1931).

⁴² Volkmann, *ibid.*, **B 10**, 161 (1930).

⁴³ Kuhn, *ibid.*, **B 30**, 356 (1935).

⁴⁴ Liquier-Milward, *Trans. Faraday Soc.*, **26**, 390 (1930).

⁴⁵ Levene and Rothen, *J. Phys. Chem.*, **34**, 2567 (1930).

expected since the absorption bands of the CO_2H and NH_2 groups are displaced towards higher frequencies in the ionized states, COO^- and NH_3^+ . For example, the direction of rotation of the lactate ion is opposite to that of the undissociated acid. The partial rotations, however, do not change their respective signs, but the rotatory dispersion of the ion is anomalous, whereas that of the acid is normal.

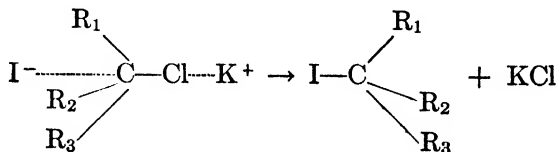
The effect of strong electrolytes has received considerable attention, particularly on the part of Clough. The older writers attributed the changes in rotation to changes in the equilibrium of two isomeric species. More recent work has shown that the effect of a series of strong electrolytes on rotation is completely analogous to that observed by Fajans and co-workers on refractivity.

Walden Inversion

It is beyond the scope of this chapter to deal at length with the discovery of Walden in 1893 that the reaction of substitution on the asymmetric carbon atom is frequently associated with an inversion of configuration, or to deal with a review of the mass of accumulated experimental material regarding this phenomenon. Although the growth of the experimental material is continuous, the nature of the phenomenon remains unsolved. Little profit would result from a review of all the theories advanced for the explanation of the mechanism of the inversion, for as yet none of them permits the prediction of the occurrence or non-occurrence of a configurational change in a special reaction of substitution. Yet it may be instructive to review some of the more recent theories and to test them in the light of some known observations.

One of the theories, although very recent, has its roots in the speculations of Werner which were further developed by Meisenheimer. In the original form the theory postulated that prior to substitution the substituting element forms an addition complex with the radical or element to be substituted, the result of the complex formation being a change in the general distortion of the molecule so that the substituting group does not enter the molecule in the position of the radical or atom to be displaced.

The following model may represent the course of a reaction of substitution resulting in a Walden inversion.



This theory had a newer development in the hands of Holmberg and more recently of Polanyi *et al.* These authors based their views on the observation of the reactivities of primary, secondary, and tertiary halides toward sodium vapor, on the one hand, and toward sodium iodide, on the other. The order of reactivity was the reverse in the second case. The authors then postulated that substitution with positive ions took place on the side of the halogen, and hence proceeded without inversion, whereas substitution with a negative ion took place on the opposite side, as in the above diagram, and hence always resulted in a Walden inversion. In harmony with this theory, Polanyi⁴⁶ and co-workers found that the rate of racemization of an alkyl iodide by iodine ions (or by iodine atoms) proceeds at a rate which is practically identical with the rate of substitution. This rate of substitution was arrived at by a comparison of the rates of substitution in related halides.

The considerations of Polanyi and co-workers received confirmation through the very elegant experiments of Hughes, Juliusburger, Masterman, Topley, and Weiss,⁴⁷ who showed that substitution of radioactive iodine for iodine in *dextro-sec.*-butyl iodide, proceeded at practically the same rate as racemization; the results of similar experiments on bromides were analogous.

Olson *et al.*⁴⁸ came to the conclusion that *every reaction of substitution is accompanied by inversion*. If an inversion does not occur, Olson accepts two substitutions in the course of the reaction. These authors studied the dynamics of the substitution of the bromine atom in bromosuccinic acid by chlorine. The reaction involves no fewer than five steps. The authors determined the rate constants of every one of these reactions and were able to calculate the rates of substitution and of racemization, obtaining results which qualitatively agreed with their theory.

It is surprising that Olson should have chosen bromosuccinic acid to test his theory when numerous substances are available in which the reaction of substitution proceeds in a simpler way.

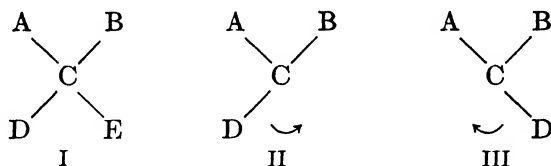
Thus Olson went further in his conclusion than Polanyi, who differentiated between positive and negative mechanisms, one proceeding without, the other with, inversion. However, even Polanyi's theory fails to explain the differences in the results of substitutions under analogous conditions in members of homologous series. It fails to explain the effect on substitution of solvent, reagent, etc.

⁴⁶ Bergmann, Polanyi, and Szabo, *Trans. Faraday Soc.*, **32**, 843 (1936).

⁴⁷ Hughes, Juliusburger, Masterman, Topley, and Weiss, *J. Chem. Soc.*, 1525 (1935).

⁴⁸ Young and Olson, *J. Am. Chem. Soc.*, **58**, 1157 (1936); Olson and Long, *ibid.*, **58**, 393 (1936).

A second of the recent theories is that of Rördam.⁴⁹ This author developed a new hypothesis of the mechanism of the Walden inversion based on the assumption that, when one of the radicals linked to the asymmetric carbon is removed, the optically active molecule oscillates between two configurations.



Thus when *E* is removed in (I), or rather nearly removed, the groups *A*, *B*, and *D* oscillate. For simplification it may be assumed that only *D* oscillates and does so between positions (II) and (III). It is self-evident that the occurrence or non-occurrence of an inversion depends on the time of substitution. If it occurs before *D* in position (II) changes into position (III), there is no inversion. The theory of Rördam postulates that, where substitution leads to two forms of opposite rotation, that form has the original configuration whose proportion increases with the increase of the concentration of the reagent. A mathematical formula was developed permitting calculation of the expected ratio of the two forms. A fair agreement was obtained between theory and experimental results.

In a qualitative way the same considerations were developed by Levene and co-workers.^{50, 51}

The relationship between the velocity of reaction and the occurrence or non-occurrence of Walden inversion was experimentally demonstrated by Ott⁵² in the action of nitrous acid on 1-phenylethylamine and on asparagin. The same author showed that the rate of reduction of phenylmethylchloroacetic acid determined the direction of rotation of the resulting hydratropic acid. The catalytic reduction proceeded with change of sign of rotation, whereas reduction with zinc and acetic acid proceeded without change of sign. This theory, however, is too elastic to predict the result in every given reaction.

According to Ingold,^{52a} occurrence or non-occurrence of an inversion in the reaction of substitution depends upon the mechanisms of the reaction. Of these he visualizes two:

⁴⁹ Rördam, *J. Chem. Soc.*, 2017 (1930).

⁵⁰ Levene and Walti, *J. Biol. Chem.*, **73**, 263 (1927).

⁵¹ Levene and Rothen, *ibid.*, **81**, 359 (1929).

⁵² Ott, *Ber.*, **68**, 1651 (1935).

^{52a} Ingold, *J. Chem. Soc.*, 236 (1935).

1. One in which the substance undergoes a primary dissociation into ions or radicals.

2. One in which the formation of an addition complex is an essential condition for the extrusion of the group replaced.

The reactions of the first type, according to Ingold, may proceed without inversion whereas those of the second are connected with inversion.

The extent to which this theory will permit prediction remains to be seen. Frequently a small change in conditions of reaction alters the result of a substitution. Some of these will be mentioned here. They may be classified into two categories: one dealing with structural conditions, and the other with external extramolecular influences.

STRUCTURAL CONDITIONS

Position in an Homologous Series. The action of hydrogen bromide on $\text{CH}_3\text{CH}(\text{OH})\text{C}_6\text{H}_5$ and on $\text{C}_2\text{H}_5\text{CH}(\text{OH})\text{C}_6\text{H}_5$ results in substitution accompanied by change of rotation. No change of rotation occurs with $\text{C}_3\text{H}_7\text{CH}(\text{OH})\text{C}_6\text{H}_5$.

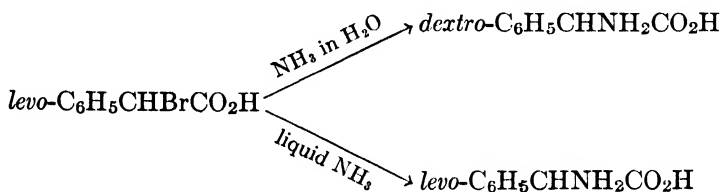
Aliphatic or Aromatic Series. The chlorination by means of thionyl chloride is accompanied in aliphatic secondary alcohols by change of rotation in the same way as with other chlorinating reagents. In the phenyl series it proceeds without change of rotation whereas other chlorinating agents act in the same way as in the aliphatic series.

Distance of the Phenyl Group from the Asymmetric Center. The result of substitution of hydroxyl by chlorine in $\text{C}_6\text{H}_5\text{CH}(\text{OH})\text{CO}_2\text{H}$ depends on the solvent. It is independent of it in $\text{C}_6\text{H}_5\text{CH}_2\text{CH}(\text{OH})\text{CO}_2\text{H}$.

INFLUENCE OF EXTERNAL CONDITIONS

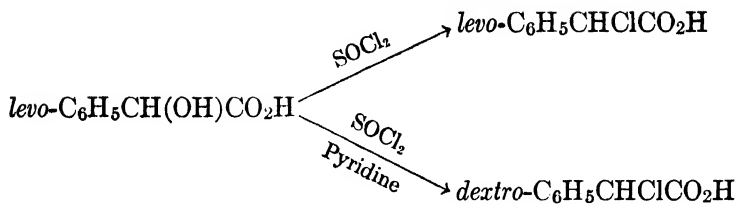
Influence of solvent.

(a) Substitution of a halogen atom by NH_2 .⁵³



⁵³ Senter and Ward, *J. Chem. Soc.*, **127**, 1847 (1925).

(b) Chlorination by SOCl_2 .⁵⁴



The effect of a tertiary base has also been observed in sugars. Thus either α - or β -glucosides are formed from *d*-acetobromoglucose, depending upon the presence of quinoline or silver carbonates in the reacting system.⁵⁵

The action of nitrous acid on *dextro*-methylphenylaminomethane produces a *dextro* carbinol when the solvent is glacial acetic acid and a *levo* carbinol when the reaction is carried out in aqueous solution.

Influence of Metallic Ion. In the monograph of Walden numerous examples are given of the differences in action of silver or other metallic ions on the results of substitution of a halogen atom by a hydroxyl group.

The effect of hydrogen-ion concentration, of neutral salts and of temperature has been recorded by many observers and has been more comprehensively studied by Holmberg⁵⁶ and by Bancroft.⁵⁷

It is noteworthy that all factors influencing the course of substitution also affect the rotations of optically active substances not subject to any chemical change. The effect on the rotation was discussed earlier and was viewed as the result of the differences in the forces of coupling of electronic vibrations in chromophoric groups located in different parts of the molecule. Is it not possible to assume that there exists some connection between the forces which affect the rotations of the substances and those which determine the steric course of a reaction of substitution? May not a rigorous investigation of such parallelisms in homologous series furnish some very valuable data? It would seem that a rational theory of Walden inversion could scarcely be expected before a theory of optical rotation is developed capable of predicting all observations on the correlation of optical rotation to chemical structure in simple substances having one asymmetric carbon atom.

⁵⁴ Kenyon, Lipscomb, and Phillips, *ibid.*, 415 (1930).

⁵⁵ Fischer and Bergmann, *Ber.*, **50**, 711 (1917).

⁵⁶ Holmberg, *Ber.*, **60**, 2194 (1927).

⁵⁷ Bancroft and Davis, *J. Phys. Chem.*, **35**, 1253 (1931).

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CHAPTER 22

THE SIGNIFICANCE OF RESONANCE TO THE NATURE OF THE CHEMICAL BOND AND THE STRUCTURE OF MOLECULES

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INTRODUCTION

The development of the quantum mechanics during the last decade has led to the clarification of many concepts previously originated by the chemist regarding valence and the nature of the chemical bond, and also to the introduction of some new ideas. Of the latter the most important is the idea of *resonance*, and especially of the *resonance of a molecule among several valence-bond structures*, which, although foreshadowed to some extent by early chemical theories, had not been clearly formulated on the basis of empirical evidence.* In this chapter we shall discuss in a systematic way the essential features of the modern conception of the chemical bond, omitting, however, all quantitative calculations, the quantum-mechanical discussion being restricted to the qualitative description of the results which have been obtained and the discussion of the physical and chemical concepts involved.

The treatment of the chemical bond and the structure of molecules given in this chapter is based largely on the fundamental concept of the shared-electron-pair bond as formulated by G. N. Lewis and developed by many investigators. A description of this development is given in Chapter 19, "Modern Electronic Concepts of Valence" (p. 1597), in which references to the earlier literature are contained.

THE ELECTRONIC STRUCTURE OF ATOMS

During the last twenty-five years a large amount of experimental information has been gathered regarding the structure of atoms, relating to the frequencies and intensities of spectral lines, the magnitudes of resonance and ionization potentials, the behavior of atoms in magnetic and electric fields, etc. This information has, after much effort, been correlated through the development of a theory which seems at present to represent in a completely satisfactory way the extranuclear electronic structure of atoms. This theory, called quantum mechanics or wave mechanics, is a refinement of the old quantum theory. It is not a complete theory of the physical world—it has not been found possible to include within it all the refinements of the theory of relativity, nor to ex-

* The idea of the resonance of molecules among several valence-bond structures, to which a vague resemblance is shown by Kekulé's theory of the benzene ring and Thiele's theory of partial valence [Thiele, *Ann.*, **306**, 87 (1899)], is much more closely approximated by Arndt's theory of intermediate stages [Arndt, Scholz, and Nachtwey, *Ber.*, **57**, 1903 (1924); Arndt, *Ber.*, **63**, 2963 (1930)] and the theory of the mesomeric state developed by the English and American organic chemists [Lowry, *J. Chem. Soc.*, 822, 1866 (1923); Lucas and Jameson, *J. Am. Chem. Soc.*, **46**, 2475 (1924); Robinson and co-workers, *J. Chem. Soc.*, 401 (1926); Ingold and Ingold, *ibid.*, 1310 (1926); see in particular Ingold, *Chem. Rev.*, **15**, 225 (1934)].

tend it to encompass electromagnetic phenomena and the structure of atomic nuclei—but in the field of atomic structure and molecular structure the very extensive agreement between deductions from quantum mechanics and the results of experiment together with the extensive experimental verification of theoretical predictions has caused most theoretical scientists to consider the theory to be generally valid.

In the following paragraphs a brief outline is given of the present views regarding the electronic structure of atoms. The statements made here without support are based upon many experimental facts, but lack of space necessitates their omission.

According to the Bohr theory the electron in the hydrogen atom in its normal state revolves about the nucleus in a circular orbit with radius

$a_0 = 0.529 \text{ \AA}$ ($1 \text{ \AA} = 1 \times 10^{-8} \text{ cm.}$)

and the constant speed $v_0 = 2.182 \times 10^8 \text{ cm. per sec.}$

The quantum-mechanical picture is similar but less definite. The state of motion of the electron is represented by an *orbital wave function* (*Eigenfunction*), ψ , obtained by solution of the Schrödinger wave equation. (Following Mulliken, the one word *orbital* will be used for a one-electron orbital wave function.) In the physical interpretation of the quantum mechanics the square of the wave function, ψ^2 , represents the *probability distribution function* for the position of the electron, such that

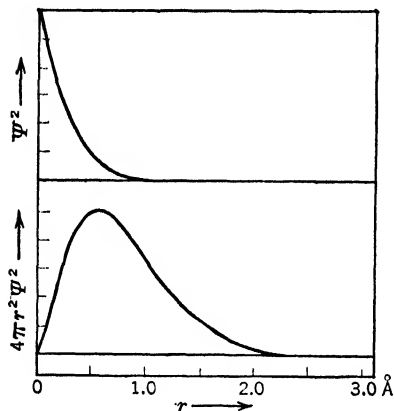


FIG. 1.—The probability functions ψ^2 and $4\pi r^2\psi^2$ for the normal hydrogen atom.

$\psi^2 dV$ is the probability that the electron be found in the volume element dV , and $4\pi r^2\psi^2 dr$ the probability that it be found between the distances r and $r + dr$ from the nucleus. These quantities are shown in Fig. 1, as calculated for the wave function

$$\Psi_{1s} = \frac{1}{\sqrt{\pi a_0^3}} e^{-r/a_0}$$

in which r is the distance from the electron to the nucleus. It is seen that the electron is not restricted to the distance a_0 from the nucleus, but that it does remain most of the time at about this distance, which is indeed the value of r at which the radial distribution function has its maximum value. Moreover, the speed of the electron is also not con-

stant, but can be similarly represented by a probability distribution function, and it is found that the root-mean-square speed has just the Bohr value v_0 . The normal hydrogen atom can accordingly be described by saying that the electron moves in and out about the nucleus, with about the speed v_0 , in such a way as to remain most of the time within a distance not much greater than a_0 . Over a period of time long enough to permit many cycles of motion of the electron the normal hydrogen atom can be described as consisting of the nucleus surrounded by a spherically symmetrical ball of negative electricity (the electron blurred by a time-exposure of its rapid motion). The exponential nature of the wave function makes it impossible for us to assign a definite radius to the atom, which fades away gradually with increasing r , but from Fig. 1 it may be said that it has a radius of around $2a_0$ (or $3a_0$), since the chance that the electron gets beyond this distance is small.

The electron itself has a spin (similar to the rotation of the earth about its own axis), and the spin can be oriented in either one of two ways (+ or -) relative to a specified direction. Only two electrons can occupy the same orbital, and these two only by having their spins opposed (*Pauli exclusion principle*). The normal helium atom consists of two electrons with opposed spins occupying the $1s$ orbital. In normal atoms containing more electrons the $1s$ orbital is always occupied in this way by two electrons, which are said in this case to constitute a *completed shell*, the *K shell*. The size (linear dimensions) of the *K shell* varies about inversely with the effective nuclear charge, the helium atom being about one-half as large as the hydrogen atom, the lithium ion Li^+ about one-third as large, and so on.

The next outer shell is the *L shell*, consisting of the four orbitals $2s$, $2p_x$, $2p_y$, and $2p_z$, of which $2s$ is somewhat more stable than the $2p$'s. In the atoms lithium to neon, electrons are introduced in these orbitals, two electrons in the same orbital having opposed spins, neon then having a completed *L shell* of eight electrons. This *electronic configuration* is represented by the symbol $1s^2 2s^2 2p_x^2 2p_y^2 2p_z^2$ or $1s^2 2s^2 2p^6$, the superscript showing the number of electrons occupying the orbital or orbitals. Here the numbers 1 and 2 (for *K* and *L* shell, respectively) give the values of the *total quantum number* n , and the letters s and p represent the values of the *azimuthal quantum number** l (s, p, d, f , etc., corresponding to $l = 0, 1, 2, 3, \dots, n - 1$).

* In the old quantum theory the azimuthal quantum number determined the eccentricity of the elliptical orbit. This interpretation is retained essentially in the quantum mechanics, the s orbital in a given shell being the most eccentric and penetrating most deeply into the core, the p orbitals next, and so on. The greater penetration into the core (the region near the nucleus) leads to greater stability, and thus gives rise to the stability sequence $ns > np > nd$, etc., indicated in Fig. 2.

In an atom or monatomic ion the electrons tend not to pair with one another (by occupying the same orbital, their spins being opposed), but instead to occupy different orbitals, keeping their spins parallel. For example, in the normal nitrogen atom there are three unpaired electrons. The two most stable orbitals, $1s$ and $2s$, are occupied by pairs, whereas the three next orbitals, $2p_x$, $2p_y$, and $2p_z$, which do not differ in stability, are occupied by one electron apiece. In oxygen the eighth electron

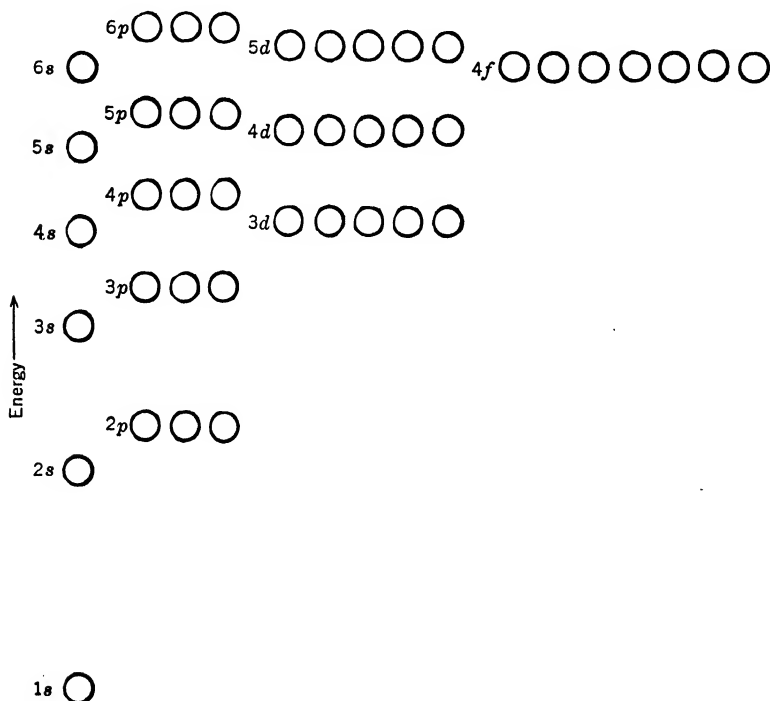


Fig. 2.—The approximate stability sequence for atomic orbitals, the lowest circle representing the most stable orbital ($1s$). Each circle represents one atomic orbital, which can be occupied either by one electron or by two electrons with opposed spins. In helium the $1s$ orbital is filled (with two electrons), in neon the $1s$, $2s$, and three $2p$ orbitals, and so on.

must pair with one of these three in order to enter the L shell, leaving only two unpaired electrons; the same process leads to one unpaired electron in fluorine and none in neon.

There are n^2 orbitals in the shell with total quantum number n , 1 in the K shell, 4 in the L , 9 in the M , 16 in the N , and so on, the numbers of electrons occupying a completed shell being thus $2n^2$. The approximate relative energy values for atomic orbitals are indicated in Fig. 2, the

most stable orbitals being the lowest. It is seen that the *M* shell is not completely filled with electrons before the *N* orbitals begin to be occupied. Instead, after the *3s* and *3p* orbitals are occupied by an "octet" of eight electrons, giving the stable argon configuration $1s^2 2s^2 2p^6 3s^2 3p^6$, electrons enter the *4s* orbitals (in potassium and calcium), and only later, in the iron-group transition elements, are the *3d* orbitals filled by their complement of ten electrons. The palladium and platinum transition elements (ten of each) correspond to filling the five *4d* and five *5d* orbitals, respectively, and the rare earths (fourteen) to filling the seven *4f* orbitals.

It must be mentioned that the stability sequence shown in Fig. 2 is not strictly applicable in all cases. In potassium and calcium the *4s*

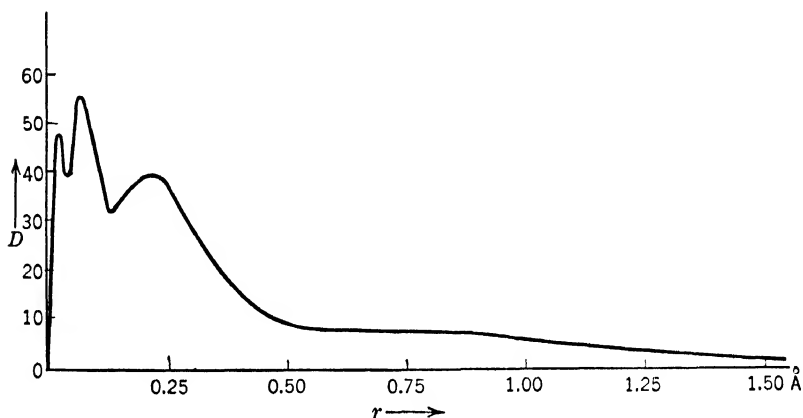


FIG. 3.—The radial electron distribution function for the rubidium ion, showing four electron shells, the outermost being not well defined. (From calculations by Hartree.)

orbital is more stable than the *3d* orbitals, and hence is occupied by electrons, whereas with increase in the atomic number (iron, cobalt, nickel) the *3d* orbitals become more stable than the *4s*. The same change in relative stability of orbitals takes place in the other transition series.

The outer shell of many stable monatomic ions consists of an octet of eight electrons in *s* and *p* orbitals (noble-gas type) or of eighteen electrons in *s*, *p*, and *d* orbitals (eighteen-shell type — Zn^{++} , etc.).

The radial electron distribution function for rubidium ion, with the configuration $1s^2 2s^2 2p^6 3s^2 3p^6 3d^{10} 4s^2 4p^6$, is shown in Fig. 3. The *K*, *L*, *M*, and *N* shells are represented by the successive humps.

ELECTROSTATIC BONDS AND COVALENT BONDS

It is convenient to consider three general classes of chemical bonds: *electrostatic bonds*, *covalent bonds*, and *metallic bonds*. This classification is not a rigorous one; for although the bonds characteristic of each of the classes have well-defined properties, especially from the structural viewpoint, the transition from one class to the other may take place gradually, permitting the existence of bonds of intermediate type (p. 1858). In other cases there may occur a discontinuity in some physical or chemical property, which may be used as a basis for classification (p. 1863). Metallic bonds will not be discussed in this chapter.

The Ionic Bond and Other Electrostatic Bonds. We say that there is a chemical bond between two atoms or groups of atoms if the forces acting between them are such as to lead to the formation of an aggregate with sufficient stability to make it convenient for the chemist to consider it as an independent molecular species. (Thus the weak van der Waals' forces between molecules are not usually considered as leading to the formation of chemical bonds.) If we can assign to each of the atoms or groups of atoms a definite electronic structure, essentially independent of the other atom or group, such that electrostatic interactions lead to strong attraction and the formation of a chemical bond, the bond is said to be an *electrostatic bond*.

The most important of these is the *ionic bond*, resulting from the Coulomb attraction of the excess electric charges of oppositely charged ions. There are ionic bonds between Na^+ and Cl^- in crystalline sodium chloride and in NaCl molecules in sodium chloride vapor.* The fluo-ferriate complex ion, $[\text{FeF}_6]^-$, consists of Fe^{+++} and F^- ions held together by ionic bonds.

In $[\text{Fe}(\text{H}_2\text{O})_6]^{+++}$, $[\text{Ni}(\text{H}_2\text{O})_6]^{++}$, $[\text{Ni}(\text{NH}_3)_4]^{++}$, and many other complexes the bonds between the central ion and the surrounding molecules are due essentially to the electrostatic attraction of the excess charge of the central ion for the permanent electric dipoles of the molecules.¹ Electrostatic bonds of this type may be called *ion-dipole bonds*. Electrostatic bonds may also result from the attraction of an ion for the induced dipole of a polarizable molecule or from the mutual interaction of the permanent electric dipoles of two molecules.

The Shared-electron-pair Bond or Single Covalent Bond. With

G. N. Lewis (1916) electronic structures such as $\text{H}:\text{H}:$, $\begin{array}{c} \text{H} \\ \vdots \\ :\text{Cl}:\text{Cl}: \\ \vdots \\ \text{H} \end{array}$, $\text{H}:\overset{\cdot\cdot}{\underset{\cdot\cdot}{\text{C}}}:\text{H}$, $\begin{array}{c} \text{H} \\ \vdots \\ :\text{C}:\text{C}: \\ \vdots \\ \text{H} \end{array}$

* For a discussion of ionic bonds in crystals see Pauling, *J. Am. Chem. Soc.*, **51**, 1010 (1929).

¹ Langmuir, *ibid.*, **41**, 868 (1919), especially pp. 930-931.

etc., are written, in which only the outer electrons are represented. Here a bond is formed between two atoms by two electrons which are held jointly by the two atoms, and which can be considered as contributing to the outer shell of each. Such a bond is called a *shared-electron-pair bond* or *single covalent bond*.

The nature of these bonds is now well understood as the result of the application of quantum mechanics, beginning with the pioneer work of Heitler and London² and culminating in the accurate treatment of the hydrogen molecule by James and Coolidge.³

A single covalent bond between two atoms *A* and *B* involves two electrons, one orbital from atom *A*, and one orbital from atom *B*. One of the electrons has positive spin and one negative spin; the stability of the bond may be considered to result from the interchange of the two electrons between the atoms *A* and *B*; that is, from *resonance* between the structures $A \uparrow \downarrow B$ and $A \downarrow \uparrow B$, the arrows indicating the orientation of the electron spins.

The energy required to separate two atoms joined by a single covalent bond is of the order of magnitude of 50,000 to 100,000 cal. per mole. The strength of the bond depends on the nature of the orbitals involved (p. 1859).

THE IDEA OF RESONANCE*

The idea of *resonance*, in its application to chemistry, is the following. If it is possible to write for a molecule (or other system) two or more electronic structures corresponding to about the same energy and satisfying certain other conditions, then no one of the structures alone can be considered to represent the normal state of the molecule, which instead is represented essentially by an average of all of them; and, moreover, the molecule is then more stable (has a smaller energy content) than it would be if it had any one of the structures alone. The molecule is described as *resonating* among various structures, and the energy stabilizing the molecule is called *resonance energy*.

(In quantum-mechanical terms, it is said that the wave function representing the normal state of the molecule is not any one of the wave functions corresponding to the various electronic structures, but is a linear combination of them.)

The principal conditions for resonance are that the structures corre-

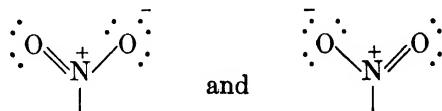
² Heitler and London, *Z. Physik*, **44**, 455 (1927).

³ James and Coolidge, *J. Chem. Phys.*, **1**, 825 (1933).

* For a more thorough discussion of this subject see Pauling and Wilson, "Introduction to Quantum Mechanics, with Applications to Chemistry," McGraw-Hill Book Co., New York (1935), Secs. 41, 46f.

spond to the same atomic arrangement (nuclear configuration) and to the same number of unpaired electrons.

The effect of the energy of the structures is the following. If two possible structures have the same energy (and satisfy the other conditions for resonance, mentioned above) the molecule resonates equally between them. For example, for the nitro group we write the two structures:



The group resonates between these two structures equally, and is thereby made more stable than either one of them. If one structure is much less stable than the other, its contribution is very small, and resonance makes the molecule only slightly more stable than the more stable of the two structures.

It has already been mentioned that the energy of a single covalent bond between two atoms *A* and *B* can be considered as the resonance energy between the two equivalent structures $A \uparrow \downarrow B$ and $A \downarrow \uparrow B$. In the following sections other applications of the idea of resonance will be discussed.

THE COVALENT BOND

The Ionic Character of Covalent Bonds. For a molecule such as HCl we write two reasonable electronic structures, $\text{H}:\ddot{\text{Cl}}:$ and $\text{H}^+:\ddot{\text{Cl}}^-$, the first corresponding to a *normal covalent bond* between the two atoms (similar to the bonds in H_2 and Cl_2) and the second to an ionic bond. Inasmuch as chlorine is electronegative with respect to hydrogen, we expect the ionic structure, although less stable than the normal covalent structure, to be not far removed from it in energy. These two structures satisfy the conditions for resonance, and the normal HCl molecule must be considered as represented by both of them. The bond is partially covalent and partially ionic, the covalent contribution being the greater. *The bond is stronger than either the normal covalent bond or the ionic bond*, as the result of the resonance energy. It is the stabilizing effect of the partial ionic character which makes covalent bonds between unlike atoms more stable than those between like atoms. A quantitative treatment of the energy of bonds in relation to the relative electronegativity of atoms has been given.⁴

⁴ Pauling, *J. Am. Chem. Soc.*, **54**, 3570 (1932); see also Mulliken, *J. Chem. Phys.*, **2**, 782 (1934); **3**, 573 (1935).

A single bond may lie anywhere between the ionic extreme and the normal covalent extreme. The former extreme is approached closely in CsF, and the latter is reached in bonds between like atoms, as in H₂. In the series of gas molecules HF, HCl, HBr, HI, there is evidence that the ionic character is large in HF (perhaps larger than the covalent character), and that it falls off rapidly in the order HCl, HBr, HI, the last having very little ionic character.

It must be pointed out that the deduction of bond type from physical properties must be made with great caution. Thus of the fluorides

	NaF	MgF ₂	AlF ₃	SiF ₄	PF ₅	SF ₆
M.P.	980°	1400°	1040°	-77°	-83°	-55°C.

those of high melting points have been described as ionic compounds and the others as covalent compounds. Actually the Al-F bond is no doubt closely similar to the Si-F bond. The abrupt change in properties between AlF₃ and SiF₄ is due to a change in atomic arrangement—in the number and distribution of the bonds rather than in their type. In NaF, MgF₂, and AlF₃ each metal atom or ion is surrounded by six fluorine atoms or ions, to which it is bonded, and each fluorine is bonded to more than one metal (six in NaF, three in MgF₂, two in AlF₃) in such a way as to make the whole crystal one giant molecule, so that fusion and vaporization can occur only through breaking these strong bonds. In SiF₄, PF₅, and SF₆ crystals there are discrete molecules, each fluorine being bonded only to the central atom; these molecules are held together only by weak van der Waals' forces, and so the substances melt and boil easily. As pointed out long ago by Kossel,⁵ this ease of fusion and vaporization would be expected for ionic molecules of high symmetry and is not sound evidence for the presence of covalent bonds. There is strong evidence, such as that mentioned above, that volatility does not depend mainly on bond type, but on the atomic arrangement and the distribution of the bonds.

Bond Orbitals. The Tetrahedral Carbon Atom. An orbital in an atom, such as the *s* and *p* orbitals indicated in Fig. 4, can be occupied by one unpaired electron or by two electrons, which form an *unshared pair*. An atomic orbital can also be involved in bond formation, the single covalent bond consisting of the shared pair of electrons occupying two atomic orbitals, one for each atom. These orbitals are conveniently called *bond orbitals*.

A simple quantum-mechanical treatment of the relation between the strengths and relative orientation of the covalent bonds formed by an

⁵ Kossel, *Z. Physik*, **1**, 395 (1920).

atom and the nature of its bond orbitals has been given.⁶ It has been seen from the foregoing discussion that the stability of a covalent bond is determined by the resonance energy of the two electrons between the two bond orbitals, one for each atom. The examination of the form of the resonance integral shows that the resonance energy increases in magnitude with increase in the *overlapping* of the two bond orbitals (the word overlapping signifying the extent to which the regions in space in which

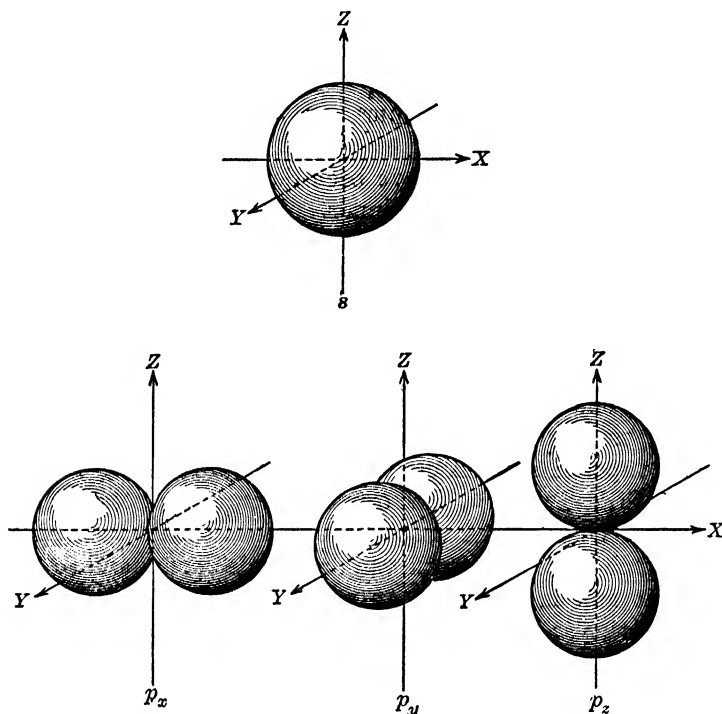


FIG. 4.—Representation of angular dependence of s and p atomic orbitals. The magnitude of each wave function, depending on orientation (polar angles ϑ and φ), is represented for each wave function by a vector drawn from the origin in the direction ϑ , φ under consideration to the surfaces shown.

the two orbital wave functions have large values coincide). Consequently it is expected that *of two orbitals in an atom the one which can overlap more with an orbital of another atom will form the stronger bond, and that, moreover, the bond formed by a given orbital will tend to lie in that direction in which the orbital is concentrated.*

The different bond orbitals of a given atom do not differ very much

⁶ Pauling, *J. Am. Chem. Soc.*, **53**, 1367 (1931); see also Slater, *Phys. Rev.*, **37**, 481; **38**, 1109 (1931), and Hultgren, *ibid.*, **40**, 891 (1932).

in their dependence on r , but they may show a great difference in their dependence on ϑ and φ , that is, in their angular distribution. This is seen from Fig. 4. The s orbital is spherically symmetrical, and so can form a bond in one direction as well as in any other, whereas the three p orbitals are concentrated along the three Cartesian axes, and will tend to form bonds in these directions.* Moreover, the p orbitals are concentrated in these directions, having a magnitude $\sqrt{3}$ times as great as the s orbital; hence (because of greater overlapping) *p bonds are stronger than s bonds*. It is convenient to call this magnitude (1.732 for p orbitals, 1 for s orbitals) the *strength of the bond orbital*.

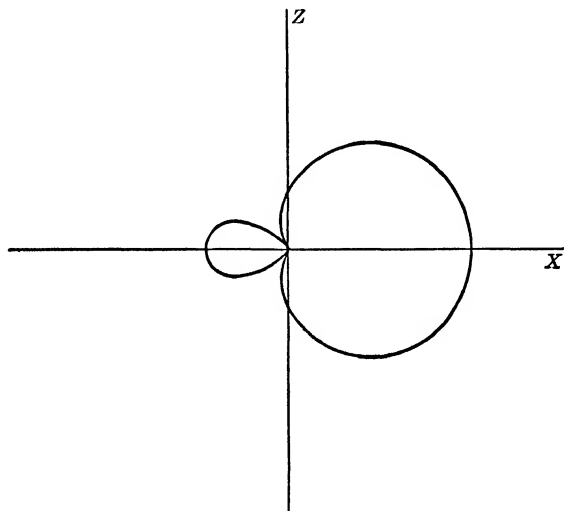


FIG. 5.—Angular dependence of a tetrahedral orbital (with cylindrical symmetry about the x axis).

The conclusion that *p bonds tend to be at right angles* is verified to some extent by experiment. In H_2S , with the electronic structure



This does not mean, however, that the carbon atom will form three p bonds at right angles and a fourth (weaker) bond in some other direction. Instead, by the process of *hybridization* (the formation of linear combinations) of the s and p orbitals four *tetrahedral bond orbitals* can be constructed; these orbitals are the best bond orbitals which can exist in

* The orientation of the axes is of course arbitrary; we should say that the bond directions for the three p orbitals are at right angles to one another.

⁷ Cross, *Phys. Rev.*, **47**, 7 (1935).

the L shell, having a strength of 2.00 as a result of great concentration in one direction (Fig. 5). The four tetrahedral bond orbitals are mutually equivalent, and they are directed towards the corners of a regular tetrahedron. The properties which these bond orbitals give to the carbon atom are just those found experimentally, which led the chemist to the concept of the tetrahedral carbon atom (p. 158).

If a first-row element forms four covalent bonds (the maximum possible, as there are only four orbitals in the L shell), these will be tetrahedrally directed, with angles $109^\circ 28'$, provided that there is no distortion arising from steric or other effects.* When only two or three bonds are formed the bond angle may lie anywhere between 90° and $109^\circ 28'$ (ignoring distortion), inasmuch as two opposing effects are operative. An unshared pair will tend to occupy the stable s orbital, leaving the p orbitals for the formation of bonds at 90° angles. On the other hand, the shared pairs strive to cause hybridization and the formation of tetrahedral bonds with use of the best bond orbitals (strength 2). That these opposing effects reach a compromise is indicated by the intermediate value, 105° , observed for the bond angle in the water molecule.⁸ The value $100^\circ \pm 3^\circ$ is also reported⁹ for OF_2 . In other oxygen compounds somewhat larger values are found,⁹ perhaps because of steric repulsion of the large attached atoms or groups: $111^\circ \pm 2^\circ$ in Cl_2O , $111^\circ \pm 4^\circ$ in dimethyl ether, and $110^\circ \pm 5^\circ$ in dioxane. The nitrogen bond angles in NH_3 and other molecules also have values of about 110° .

Other atoms (Ni^{II} , Pd^{II} , Pt^{II} , Cu^{II} , Au^{III}) can form four covalent bonds directed toward the corners of a square, using hybrid bond orbitals formed from one d , one s , and two p orbitals. Atoms such as Fe^{II} , Fe^{III} , Co^{III} , Pd^{IV} , Pt^{IV} , etc., can form six covalent bonds directed toward the corners of an octahedron, using hybrid bond orbitals formed from two d , one s , and three p orbitals.

It is to be emphasized that the quantum-mechanical treatment given above is neither rigorous nor unique. Most of the problems of chemistry are so complicated that they can be attacked in practice only through extreme simplification. The simplifying assumptions can be chosen in any one of a number of ways. Of these ways two in particular are especially reasonable; these correspond to the two general treatments which have been used to the largest extent in the treatment of the

* Electron-diffraction studies [Sutton and Brockway, *J. Am. Chem. Soc.*, **57**, 473 (1935)] have shown that the Cl-C-Cl angles in methylene chloride and chloroform have the value $111^\circ \pm 2^\circ$, only slightly different from the tetrahedral angle.

⁸ Mecke, *Z. Physik*, **81**, 313 (1933); Baumann and Mecke, *ibid.*, **81**, 445 (1933).

⁹ Sutton and Brockway, *J. Am. Chem. Soc.*, **57**, 473 (1935).

electronic structure of molecules, called the *valence-bond method* and the *molecular-orbital method*. Of these two methods the former is the more closely related to the familiar concepts of chemistry, and for this reason the discussion will be restricted to it. Confidence in the results of its application, which might be shaken by realization of its approximate character, is reinforced strongly by the fact that essentially the same results are obtained by application of the method of molecular orbitals.*

The Magnetic Criterion for Bond Type.¹⁰ It has been mentioned (p. 1859) that discontinuities in physical properties sometimes cannot be relied on as indicating a discontinuity in bond type. For certain substances, however, definite evidence regarding the bond type can be obtained by the observation of one property of the molecule, its magnetic moment.

The complex ions $[\text{FeF}_6]^-$ and $[\text{Fe}(\text{CN})_6]^-$ may be considered. If the bonds connecting the iron atom to the six surrounding groups are ionic, these complexes contain the ferric ion, Fe^{+++} , with twenty-three electrons. Of these electrons eighteen occupy the nine most stable orbitals, and the remaining five the five $3d$ orbitals, the electron configuration being $1s^2 2s^2 2p^6 3s^2 3p^6 3d^5$. Electrons in atoms or monatomic ions avoid pairing; hence the five $3d$ electrons distribute themselves among the five $3d$ orbitals without pairing, as indicated in Fig. 6. Now each unpaired electron makes a large contribution to the magnetic moment of the complex, because of its spin, so that a complex ion $[\text{FeX}_6]^-$ containing ionic bonds would have a very large magnetic moment, and a substance containing it would be strongly paramagnetic.

On the other hand, if the iron atom is attached to the six groups by octahedral covalent bonds two of the $3d$ orbitals will be involved in bond formation, together with the $4s$ and the three $4p$ orbitals (Fig. 6), and the five $3d$ electrons will be forced into the three remaining $3d$

* The following references relate to the development of the two principal methods of treatment of the electronic structure of molecules. Valence-bond method: Heitler and London, *Z. Physik*, **44**, 455 (1927); Heitler, *ibid.*, **46**, 47; **47**, 835 (1928); **51**, 805 (1929); London, *ibid.*, **46**, 455; **50**, 24 (1928); Pauling, *Proc. Natl. Acad. Sci. U.S.*, **14**, 359 (1928); *Chem. Rev.*, **5**, 173 (1928); Slater, *Phys. Rev.*, **37**, 481; **38**, 1109 (1931); Pauling, *J. Am. Chem. Soc.*, **53**, 1367 (1931). Molecular-orbital method: Burrau, *Kgl. Danske Videnskab. Selskab. Math.-fys. Medd.*, **7**, 1 (1927); Lennard-Jones, *Trans. Faraday Soc.*, **25**, 668 (1929); Hund, *Z. Physik*, **51**, 759 (1928); **63**, 719 (1930); **73**, 1, 565 (1931); **74**, 429 (1932); Herzberg, *ibid.*, **57**, 601 (1929); Mulliken, *Chem. Rev.*, **9**, 347 (1931); *Phys. Rev.*, **40**, 55; **41**, 49, 751 (1932); **43**, 279 (1933); *Rev. Modern Phys.*, **4**, 1 (1932); *J. Chem. Phys.*, **1**, 492 (1933); **3**, 375, 506, 514, 517, 564, 573, 586, 635 (1935). The problem of directed valence is discussed in the following papers, in addition to those already mentioned: Van Vleck, *J. Chem. Phys.*, **1**, 177, 219 (1933); **2**, 20, 297 (1934); Penney, *Proc. Roy. Soc. (London)*, **A144**, 166 (1934); *Proc. Phys. Soc. (London)*, **46**, 333 (1934); Penney and Sutherland, *J. Chem. Phys.*, **2**, 492 (1934); *Trans. Faraday Soc.*, **30**, 898 (1934).

¹⁰ Pauling, *J. Am. Chem. Soc.*, **53**, 1367 (1931), and references given there.

orbitals, only one remaining unpaired. This will give rise to a relatively small magnetic moment.

The experimentally determined moment for $[\text{FeF}_6]^-$ corresponds accurately to five unpaired electrons, and that for $[\text{Fe}(\text{CN})_6]^-$ to one; hence in the fluoferriate ion the bonds are essentially ionic and in the ferricyanide ion they are covalent.

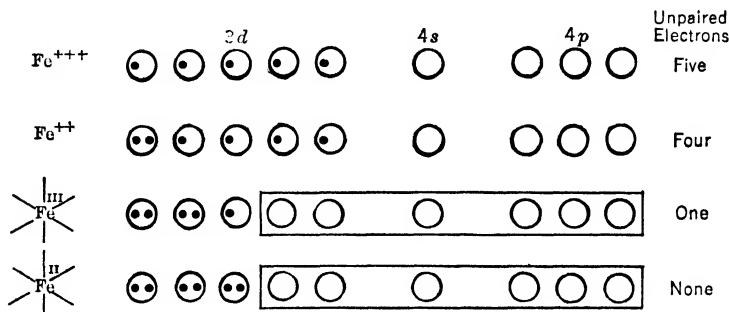
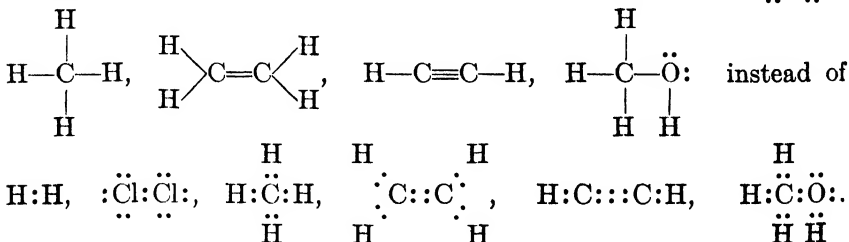


FIG. 6.—Occupancy of orbitals in iron complexes, each large circle representing an orbital and each small circle an electron. The circles enclosed in the rectangles are involved in covalent bond formation.

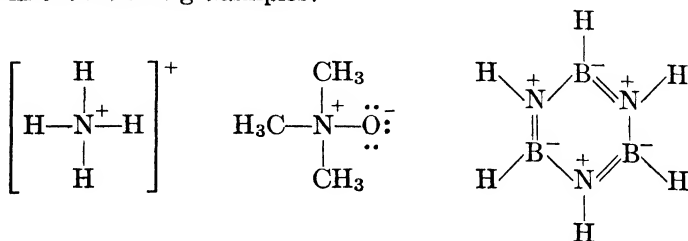
Multiple Bonds. The formal requirements for a double covalent bond between two atoms are the same as for two single covalent bonds; namely, the bond involves four electrons and four bond orbitals, two for each atom. A triple bond involves six electrons and six bond orbitals, three for each atom. Thus a first-row atom can form (with its four L orbitals) a maximum of four single covalent bonds, two single and one double, two double, or one single and one triple.

In writing electronic structures it is often convenient to use the customary valence-bond dashes, only the outer unshared electrons being represented by dots, as, for example, $\text{H}-\text{H}$, $:\ddot{\text{Cl}}-\ddot{\text{Cl}}:$,



Moreover, it may also be convenient to represent the *formal charges* of the atoms by means of small plus and minus signs, the formal charge of an atom for a given electronic structure being calculated by assigning to

that atom all its unshared electrons and one-half of the electrons which it shares with other atoms. Formal charges calculated in this way are shown in the following examples:



It must be recognized that these charges do not represent accurately the charge distribution for the molecule, inasmuch as such effects as polarization, partial ionic character of bonds, etc., are also of significance; but the formal charges are probably the expression of the most important effect.

In the discussion the terms *coördinate covalency* and *semi-polar double bonds* have not been used. The six single covalent bonds between C and Fe in $[\text{Fe}(\text{CN})_6]^-$, for example, are sometimes called coordinate covalent bonds, on the basis of the supposition that this complex is formed from Fe^{+++} and 6 $(\text{CN})^-$, the latter ions providing all the electrons for the bonds. These covalent bonds, once formed, do not differ in any way from other covalent bonds, however, and there seems to be no need for attempting to differentiate the C—Fe bond from the C—C bond in $\text{H}_3\text{C—CN}$, say, by the use of a different name. Similarly in trimethylamine oxide the bond between N and O is sometimes called a semi-polar double bond. This nomenclature may be convenient at times, the two atoms being actually held together by a single covalent bond and by an ionic bond (the electrostatic interaction of their formal charges); the use of a special symbol for the semi-polar double bond is unnecessary if the formal charges are shown in the structural formula.

The One-electron Bond and the Three-electron Bond.¹¹ The simplest molecules in which the one-electron bond and the three-electron bond occur are the hydrogen molecule-ion, H_2^+ , and the helium molecule ion, He_2^+ , respectively. The hydrogen molecule-ion consists of two protons (for each of which there is only one stable orbital, $1s$) and one electron. The two structures $\text{H} \cdot \text{H}^+$ and $\text{H}^+ \cdot \text{H}$, in which the electron occupies first one and then the other $1s$ orbital, are equivalent, and so correspond to equal energy, satisfying the condition for resonance. The system may be expected to resonate between these two

¹¹ Pauling, *ibid.*, **53**, 3225 (1931).

structures and thereby to be stabilized, forming a bond which we call the one-electron bond. This bond is only about one-half as strong as a shared-electron-pair bond, the dissociation energy of H_2^+ being 60,800 cal. per mole, as compared with 102,600 cal. per mole for H_2 .

For He_2^+ , with a 1s orbital for each nucleus and three electrons, there are also two equivalent structures, $\text{He} : \cdot\text{He}^+$ and $\text{He}^+ : \cdot\text{He}$, between which there is resonance, leading to the formation of a three-electron bond. This bond too is only about one-half as strong as a shared-electron-pair bond, the dissociation energy of He_2^+ being about 58,000 cal. per mole.

For the formation of a one-electron bond, an electron-pair bond, or a three-electron bond between two atoms there are needed two bond orbitals, one for each atom, and one, two, or three electrons, respectively. As mentioned above, a one-electron or three-electron bond is only about one-half as strong as an electron-pair bond. There is another difference in properties which causes the one-electron and three-electron bonds to be of only minor importance. An electron-pair bond can be formed between any two atoms, the conditions for resonance being always satisfied. On the other hand, the structures $\text{A} \cdot \text{B}$ and $\text{A} : \text{B}$ (or $\text{A} : \cdot \text{B}$ and $\text{A} \cdot : \text{B}$) in general will not have approximately equal energy, and so will not satisfy the energy condition for resonance; only if A and B are atoms of the same element or are of such a nature as to cause the two structures to have nearly the same energy (as for two atoms adjacent in the periodic table) will resonance occur and a one-electron or three-electron bond be formed.

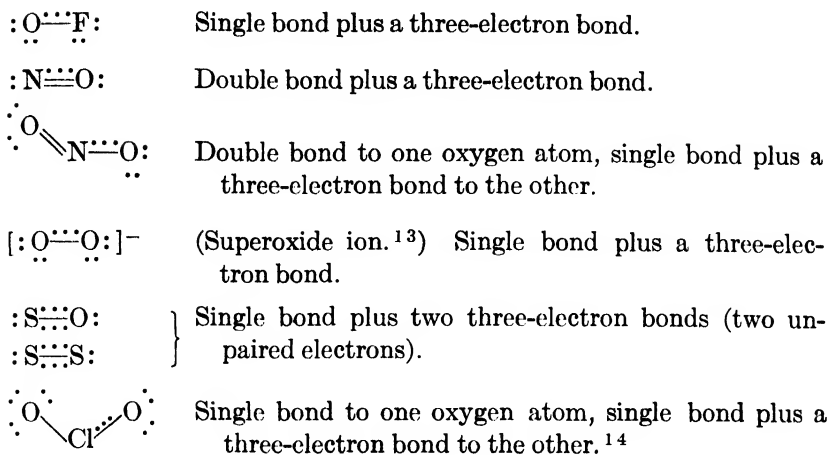
It is probable that the one-electron bond occurs in the boron hydrides,

$$\begin{array}{c} \text{H} \quad \text{H} \\ \vdots \quad \vdots \\ \text{B}_2\text{H}_6 \text{ having the electronic structure } \text{H} : \ddot{\text{B}} : \ddot{\text{B}} : \text{H}, \text{ in which two of the} \\ \vdots \quad \vdots \\ \text{H} \quad \text{H} \end{array}$$

boron-hydrogen bonds are one-electron bonds.¹²

The three-electron bond seems to occur in several molecules, between like atoms (as in He_2^+) or atoms which are adjacent to each other in the periodic table, and so are sufficiently alike to permit the resonance stabilizing this bond. Molecules and complexes containing this bond include OF , NO , NO_2 , O_2^- , O_2 , SO , S_2 , and ClO_2 , to which are assigned the following structures, using — and = for the single and double covalent bonds, and . . . for the three-electron bond.

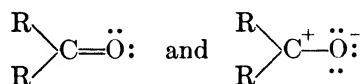
¹² Sidgwick, "The Electronic Theory of Valency," Oxford University Press, Oxford (1929), p. 103. For a more detailed discussion of the structure of the boron hydrides, see Bauer and Pauling, *J. Am. Chem. Soc.*, **58**, 2403 (1936), and Bauer, *ibid.*, **59**, 1096 (1937).



THE RESONANCE OF MOLECULES AMONG SEVERAL VALENCE-BOND STRUCTURES

The Meaning of Valence-bond Formulas. By a formula containing the symbol C—C, representing a single covalent bond between two carbon atoms, it is meant that the bond between the two atoms is essentially the same as in ethane or in diamond; in quantum-mechanical language, the same wave function (or part of a wave function) would be used for the molecule under discussion as for ethane or diamond. Similarly C=C represents a bond as in ethylene, C≡C one as in acetylene, and so on.

[The symbol C=O is usually used to represent a double bond with about the same ionic character as in acetone. This ionic character arises in large part from resonance between the electronic structures



and it is sometimes (though not usually) convenient to give this resonance explicit discussion.]

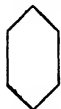
It is found empirically that many molecules are of such a nature that for each a single valence-bond formula can be written which represents the structure and properties of the molecule in a satisfactory way. Thus for these molecules the symbol C—C in the formula assigned means that the carbon-carbon bond has the chemical properties which have come

¹³ Neuman, *J. Chem. Phys.*, **2**, 31 (1934); Kassatochkin and Kotow, *ibid.*, **4**, 458 (1936).

¹⁴ Brockway, *Proc. Natl. Acad. Sci. U.S.*, **19**, 303, 868 (1933).

to be associated with a single bond between two carbon atoms, that the internuclear distance of the two atoms is 1.54 \AA , that the Hooke's-law force constant has the single-bond value, and so on.

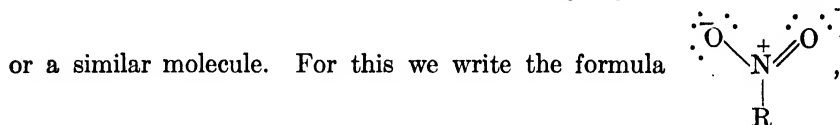
The Structure of Simple Molecules. To many molecules, however, it is impossible to assign a single valence-bond structure which satisfactorily represents the molecule. Under these circumstances some new concepts and symbols might be introduced (for example, writing



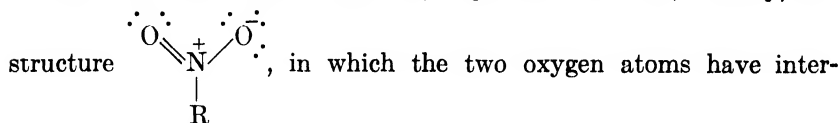
for benzene, without attempting to interpret this in terms of single

and double bonds). An alternative procedure, which has been found to be illuminating and practicable, is to assign to a molecule of this type more than one valence-bond structure, these structures all contributing to the normal state of the resonating molecule. In quantum-mechanical language, it is said that the wave function for the molecule is formed by linear combination of the wave functions corresponding to the valence-bond structures involved. The properties of the molecule are then those corresponding to the various valence-bond structures, cognizance being taken also of the extra stability resulting from the resonance itself.

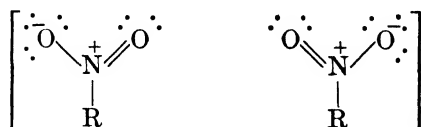
As an example let us consider the nitro group in nitromethane



using all four *L* orbitals of nitrogen for bonds. However, there is another structure which is entirely equivalent to this, namely, the



changed their roles. The two structures satisfy the conditions for resonance (they correspond to nearly the same nuclear configuration), and since they are equivalent they must contribute equally to the structure of the molecule. The molecule might then be represented by enclosing both formulas in brackets:

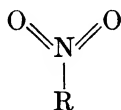


This is rather clumsy, however, and since it is evident that both equiva-

lent structures must be considered only one is usually written, it being understood that resonance with the other occurs.

It is to be emphasized again that in writing these two valence-bond structures for the molecule and saying that it resonates between them an effort is being made to extend the valence-bond picture to molecules to which it is not strictly applicable. This is not required but is chosen as a method in the hope of obtaining a satisfactory description of these unusual molecules, permitting the correlation (and "understanding") of the results of experiments on their chemical and physical properties and allowing predictions to be made in the same way as for molecules to which a single valence-bond structure can be assigned. The substance such as nitromethane does not consist of tautomeric molecules, some with one and some with the other of the two valence-bond structures written above. Instead, all the molecules have the same electronic structure, this being a structure which cannot be represented satisfactorily by any one valence-bond structure, but which is reasonably well represented by the two structures given above. The properties of the molecule are essentially those expected for an average of the two valence-bond structures, except for the stabilizing effect of the resonance energy. In nitromethane the two N—O bonds are equivalent. Each is a bond of a type intermediate between a double and a single bond; it is found experimentally that the properties of such a bond (interatomic distance, force constant) are determined mainly by the stronger of the bonds provided by the individual structures, the N—O bonds in the nitro group having properties close to those of a double bond.

It might well be asked by the chemist whether it is not then wise to write for nitromethane the valence bond-structure



which gives a satisfactory representation of the properties of the N—O bonds. It does not seem wise to do this, for the following reasons. There are strong theoretical arguments showing that the maximum number of covalent bonds which a nitrogen atom (or other first-row atom) can form is four; the structure under discussion suggests that five covalent bonds can be formed. Moreover, the structure under discussion provides little stereochemical information—a prediction could not be made as to whether the groups attached to the nitrogen atom are coplanar or not—whereas the resonating structure combined with the stereochemical knowledge of the tetrahedral nitrogen atom permits the

conclusion that the bonds are coplanar and that the O—N—O bond angle has approximately the single bond-double bond value $125^{\circ} 16'.$ *

The assignment of a resonating structure to a molecule can sometimes be made on the basis of theoretical arguments, as in the case of the nitro group just discussed, for which the two reasonable valence-bond structures are equivalent. In general, such an assignment should be supported by experimental evidence, such as that provided by chemical properties, resonance energies (as obtained from thermochemical data, p. 1874), interatomic distances,¹⁵ force constants of bonds obtained from Raman and infra-red spectra, dipole moments (p. 1712),¹⁶ etc. If the reasonable valence-bond structures are not equivalent, knowledge of the magnitudes of the contributions of different structures to the actual structure of the molecule can be obtained from such data. Thus for carbon dioxide it is customary to write the structure $:\ddot{\text{O}}=\text{C}=\ddot{\text{O}}:$; however, the observed interatomic distances show definitely that the structures $:\ddot{\text{O}}=\text{C}-\ddot{\text{O}}:$ and $:\ddot{\text{O}}-\text{C}=\ddot{\text{O}}:$ contribute to just about as great an extent as the double-bonded structure, and this conclusion is supported by the resonance energy (the great thermodynamic stability of the molecule) and the force constants of the bonds. For nitrous oxide, on the other hand, the force constants¹⁷ and interatomic distances¹⁸ show resonance between the two structures $:\ddot{\text{N}}=\ddot{\text{N}}=\ddot{\text{O}}:$ and $:\text{N}=\ddot{\text{N}}-\ddot{\text{O}}:$, the structure $:\ddot{\text{N}}-\ddot{\text{N}}=\ddot{\text{O}}:$ not contributing.

On p. 1867 the structures $\ddot{\text{O}}=\ddot{\text{N}}-\ddot{\text{O}}:$ and $\ddot{\text{O}}-\ddot{\text{Cl}}-\ddot{\text{O}}:$ were assigned to NO_2 and ClO_2 respectively. Each of the molecules, of course, actually resonates between such a structure and the equivalent one in which the roles of the two oxygen atoms are interchanged.

For the carbon monoxide molecule the two structures $:\text{C}=\ddot{\text{O}}:$ and $:\ddot{\text{C}}=\text{O}:$ have been suggested. Actually both of these contribute to the structure, which we write as $\{:\ddot{\text{C}}=\text{O}:, :\text{C}=\ddot{\text{O}}:\}$, the bond resonating between a double and triple covalent bond. The study of energy

* This has been verified by experiment, the value $127^{\circ} \pm 3^{\circ}$ being found: Brockway, Beach, and Pauling, *J. Am. Chem. Soc.*, **57**, 2693 (1935).

¹⁵ Pauling, *Proc. Natl. Acad. Sci., U. S.*, **18**, 293 (1932).

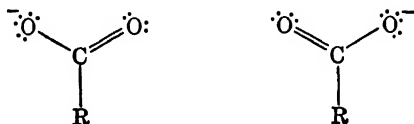
¹⁶ Sutton, *Trans. Faraday Soc.*, **30**, 789 (1934).

¹⁷ Plyler and Barker, *Phys. Rev.*, **38**, 1827 (1931).

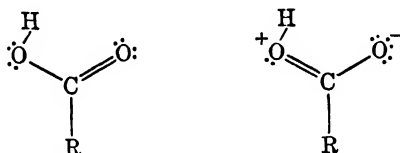
¹⁸ Pauling, *Proc. Natl. Acad. Sci. U.S.*, **18**, 498 (1932).

relations¹⁹ indicates that these structures are both important, with $\text{:C}\equiv\text{O:}^+$ contributing to a somewhat larger extent than $\text{:C}=\ddot{\text{O}}\text{:}$.

The carboxylic ions resonate between the two structures

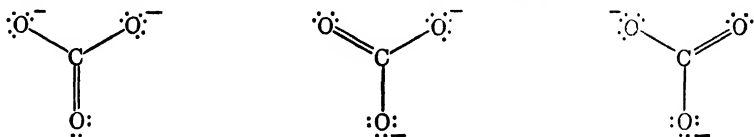


which contribute equally because of their equivalence. In the carboxylic acids the two structures



are no longer equivalent, the second contributing less than the first, and the stabilizing effect of resonance being less than for the ion. This change in resonance energy, with the ion more stable than the acid, tends to assist in detaching the proton, and so gives these acids their large acid strength. The same result can be seen from another argument. The second of the structures written for the acid makes the oxygen atom to which the proton is attached positive in sign; it accordingly repels the proton, and so increases the acid strength.*

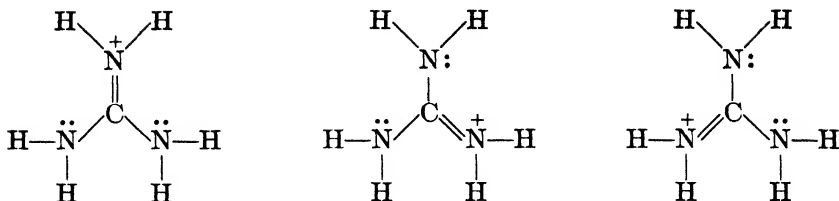
Single bond-double bond resonance also occurs in the carbonate ion, the nitrate ion, urea, guanidine, the acid amides, and similar compounds, the carbonate ion resonating among the following structures:



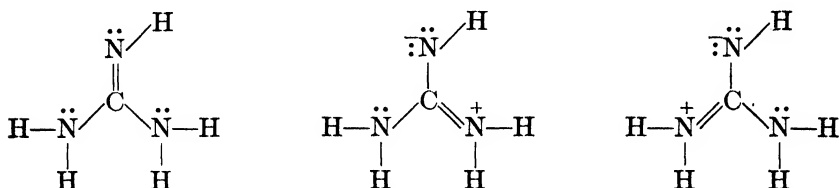
As another illustration of the application of the concept of resonance to the chemical properties of substances let us discuss the basic strengths of guanidine and substituted guanidines. The fact that guanidine is a strong base can be accounted for by either one of the two closely related arguments used above for the carboxylic acids. The guanidinium ion resonates among the three structures

¹⁹ See Pauling, *J. Am. Chem. Soc.*, **54**, 988 (1932).

* An interesting series of investigations of the effect of resonance on the acid strengths of substituted boric acids and other substances has been carried out by Branch and his collaborators: Yabroff, Branch, and Almqvist, *J. Am. Chem. Soc.*, **55**, 2935 (1933); Branch, Yabroff, and Bettman, *ibid.*, **56**, 937 (1934); Branch and Yabroff, *ibid.*, **56**, 2568 (1934).



which are all equivalent, whereas guanidine itself resonates among the three structures

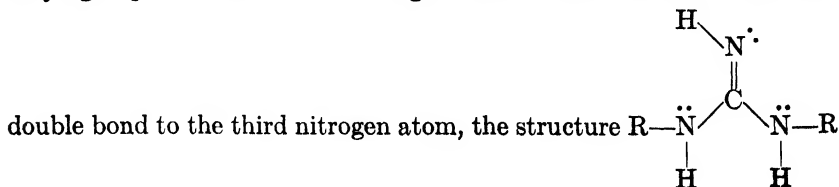


which are not equivalent, the first being more stable than the other two (it is the structure usually considered alone by the chemist) and hence contributing more to the actual state of the molecule, resonance to the other two being less important. In consequence the ion is stabilized by resonance more than the molecule, and the basic strength of the substance is increased by resonance.

It may be predicted that monoalkyl-substituted and N,N -dialkyl-substituted guanidines are weaker bases than guanidine itself, for the following reason. The replacement of one or two hydrogens of an —NH_2 group by alkyl radicals tends to prevent the double bond from swinging to this group, because carbon is more electronegative than hydrogen and hence tends to cause the adjacent nitrogen atom not to assume a positive charge. In consequence resonance of the double bond is to some extent restricted to the two other nitrogen atoms. This causes a decrease in the basic strength towards that characteristic of an imidine, the decrease

being about twice as great for $\text{HNC} \begin{matrix} \text{NH}_2 \\ \text{NR}_2 \end{matrix}$ as for $\text{HNC} \begin{matrix} \text{NH}_2 \\ \text{NHR} \end{matrix}$. A very

much larger effect is expected for the N,N' -dialkylguanidines. The alkyl groups on two of the nitrogen atoms would tend to force the



being more important than the other two. This nitrogen atom would hence have little tendency to add a proton, and the substance would be a

weak base. The tetraalkylguanidines $\text{HNC} \begin{array}{l} \nearrow \text{NR}_2 \\ \searrow \text{NR}_2 \end{array}$ would be still

weaker bases, approaching the non-resonating imines still more closely. On the other hand, the N,N',N''-trialkylguanidines may be expected to be about as strong bases as guanidine itself, inasmuch as the conditions for resonance in this molecule and its symmetrical ion are the same as for guanidine itself and its ion. These various conclusions are in agreement with the available experimental data;²⁰ guanidine, the monoalkylguanidines, N,N-dimethylguanidine, and N,N',N''-trimethylguanidines are strong bases, whereas the N,N'-dialkylguanidines are weak.

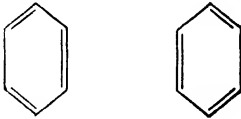
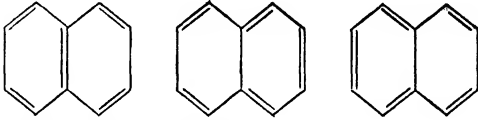
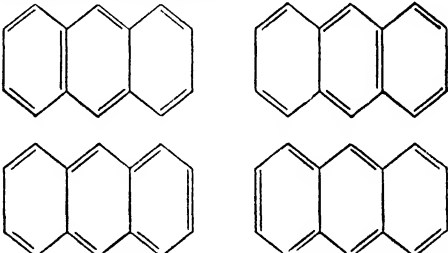
Empirical Resonance Energies. Thermochemists have often attempted to assign constant energy values to the bonds in molecules in such a way that the total energy of formation of a molecule from separated atoms could be expressed as a sum of bond energies. It is found that by restricting the discussion to molecules to each of which a single valence-bond structure can be confidently assigned this program can be carried out with considerable success; a table of bond energies can be constructed with which energies of formation of non-resonating molecules reliable to a few thousand calories can be calculated.

On applying this table to resonating molecules it is found that *the actual energy of formation of the molecule invariably is greater than the calculated value*; that is, the molecule is actually more stable than it would be if it had the valence-bond structure assumed for it in making the bond-energy calculation.* This result is the one required by the quantum mechanics, according to which resonance always exerts a stabilizing action on the molecule. The difference between the observed energy of formation (obtained from heats of combustion or other thermochemical data) and the value calculated by bond energies for an assumed valence-bond structure is an *empirical value of the resonance energy* of the mole-

²⁰ Davis and Elderfield, *ibid.*, **54**, 1499 (1932).

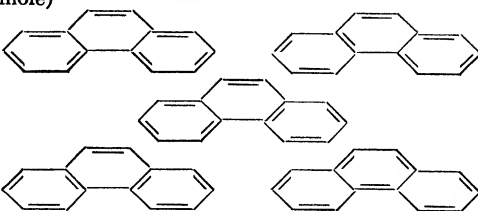
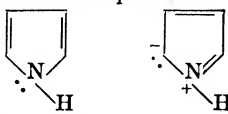
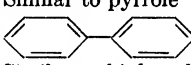
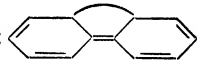
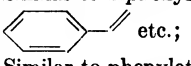
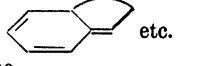
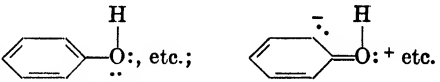
* See Pauling and Sherman, *J. Chem. Phys.*, **1**, 606 (1933), for the details of this treatment. In calculating resonance energies it is for convenience only that the thermochemical data are converted into energies of formation of molecules from separated atoms; the same results can be obtained by dealing directly with heats of formation from elementary substances in their standard states or with heats of hydrogenation reactions or other reactions. Many important results regarding resonance energies in unsaturated and aromatic compounds have been obtained recently by Kistiakowsky and his collaborators by the direct measurement of heats of hydrogenation [Kistiakowsky, Ruhoff, Smith, and Vaughan, *J. Am. Chem. Soc.*, **58**, 137, 146 (1936)]. The values found in this way are in general agreement with the less accurate values, obtained from heats of combustion, given in Table I, the values of the resonance energy found in these two ways for benzene, for example, being 36,000 and 39,400 cal. per mole, respectively.

TABLE I
EMPIRICAL VALUES OF RESONANCE ENERGY

Substance	Resonance energy (in cal. per mole)	The principal resonating structures *
CO	58,000	$\text{:C}=\ddot{\text{O}}:$, $\text{:}\ddot{\text{C}}\equiv\text{O}^+$
CO ₂	31,600	$\text{:}\ddot{\text{O}}=\text{C}=\ddot{\text{O}}:$, $\text{:}\ddot{\text{O}}\equiv\text{C}-\ddot{\text{O}}^-$, $\text{:}\ddot{\text{O}}-\text{C}\equiv\text{O}^+$
SCO	19,400	Same as for CO ₂
CS ₂	10,600	Same as for CO ₂
RNCO (R=CH ₃ , C ₂ H ₅)	6,600	$\text{R}:\ddot{\text{N}}=\text{C}=\ddot{\text{O}}:$, $\text{R}:\ddot{\text{N}}\equiv\text{C}-\ddot{\text{O}}^-$, $\text{R}:\ddot{\text{N}}^--\text{C}\equiv\text{O}^+$
RCO ₂ H	27,600	$\text{R}-\text{C}\begin{smallmatrix} \nearrow \ddot{\text{O}}: \\ \searrow \ddot{\text{O}}-\text{H} \end{smallmatrix}$, $\text{R}-\text{C}\begin{smallmatrix} \nearrow \ddot{\text{O}}^- \\ \searrow \text{O}^+-\text{H} \end{smallmatrix}$
RCONH ₂	21,000	$\text{R}-\text{C}\begin{smallmatrix} \nearrow \ddot{\text{O}}: \\ \searrow \text{N}^+-\text{H} \end{smallmatrix}$, $\text{R}-\text{C}\begin{smallmatrix} \nearrow \ddot{\text{O}}^- \\ \searrow \text{N}^+-\text{H} \end{smallmatrix}$
CO(NH ₂) ₂	36,600	$\begin{smallmatrix} \text{H} & \text{H} & \text{H} & \text{H} \\ & & & \\ \text{N} & & \text{N} \\ & & \\ \text{C} \\ \\ \text{O} \end{smallmatrix}$, $\begin{smallmatrix} \text{H} & \text{H} & \text{H} & \text{H} \\ & & & \\ \text{N} & & \text{N}^+ \\ & & \\ \text{C} \\ \\ \text{O}^- \end{smallmatrix}$, $\begin{smallmatrix} \text{H} & \text{H} & \text{H} & \text{H} \\ & & & \\ \text{N}^+ & & \text{N} \\ & & \\ \text{C} \\ \\ \text{O}^- \end{smallmatrix}$, $\begin{smallmatrix} \text{H} & \text{H} & \text{H} & \text{H} \\ & & & \\ \text{N} & & \text{N} \\ & & \\ \text{C} \\ \\ \text{O}^- \end{smallmatrix}$
R ₂ CO ₂	41,600	Same as for urea
Benzene	39,400	
Naphthalene	74,600	
Acenaphthene	71,000	Same as for naphthalene
Anthracene	104,700	

* In each case the resonance energy is calculated relative to the first of the structures given.

TABLE I—Continued

Substance	Resonance energy (in cal. per mole)	The principal resonating structures
Phenanthrene	110,000	
Pyridine	43,100	Same as benzene
Quinoline	69,400	Same as naphthalene
Pyrrole	22,600	 etc.
Furan	21,400	Same as pyrrole
Thiophene	31,000	Same as pyrrole
Indole	54,000	Similar to pyrrole
Carbazole	91,000	Similar to pyrrole
Biphenyl	8,000†	 etc.;  etc.
1,3,5-Triphenylbenzene	25,000†	Similar to biphenyl
Phenylethylene	6,700†	 etc.;  etc.
Stilbene	15,400†	Similar to phenylethylene
Phenylacetylene	10,400†	" " "
Benzonitrile	4,900†	" " "
Benzoic acid	4,200†	" " "
Benzaldehyde	3,500†	" " "
Acetophenone	7,100†	" " "
Benzophenone	10,400†	" " "
Phenol	6,700†	
Aniline	4,400†	Same as phenol

† Extra resonance energy, not including benzene resonance.

cule, which resonates between the structure assumed and other structures.

Some empirical resonance energy values are given in Table I. It is seen that the values support the statements made in the preceding section regarding the structure of some simple molecules. For carbon monoxide, resonance with the structure $\text{:C}\equiv\text{O:}$ stabilizes the molecule to the extent of 58,000 cal. per mole relative to the structure $\text{:C}=\ddot{\text{O}}\text{:}$; if this resonance did not occur the substance would not be thermodynamically stable. The observed resonance energy of 31,600 cal. per mole for

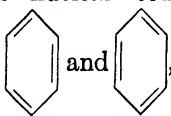
carbon dioxide shows that the structure $\text{:}\ddot{\text{O}}=\text{C}=\ddot{\text{O}}\text{:}$ for this molecule does not alone represent the molecule satisfactorily. The most reasonable structures to provide this resonance energy are $\text{:}\ddot{\text{O}}\equiv\text{C}-\ddot{\text{O}}\text{:}^+$ and $\text{:}\ddot{\text{O}}-\text{C}\equiv\text{O}\text{:}^+$, and as mentioned above, it has been verified by arguments based on interatomic distances that these structures are almost as important as the first for this molecule. Resonance of this type is much less important in carbon disulfide.²¹

In the carboxylic acids and the acid amides the resonance of the double bond between two positions gives rise to a resonance energy of about 25,000 cal. per mole; and in urea and the esters of carbonic acid resonance of the double bond among three positions leads to a resonance energy of about 40,000 cal. per mole, a reasonable value in comparison with the foregoing one.

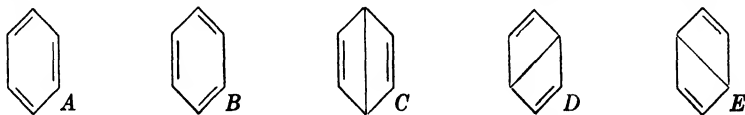
The remaining values in the table will be discussed in later sections.

THE STRUCTURE OF BENZENE AND OTHER AROMATIC MOLECULES

The Structure of Benzene (p. 55). The benzene molecule is known from electron and x-ray diffraction studies to be a plane, the six carbon atoms lying at the corners of a regular hexagon 1.39 Å on edge. This nuclear configuration is compatible with the Kekulé

structures, , which the chemist immediately writes as the

most reasonable. These two structures do indeed make the largest contributions to the structure of the normal benzene molecule. The detailed investigation* of the problem has shown that the resonance between the two Kekulé structures stabilizes the molecule to the extent of about 31,000 cal. per mole; in addition the less stable structures†



²¹ Cross and Brockway, *J. Chem. Phys.*, **3**, 821 (1935).

* Of the two general quantum-mechanical methods which have been applied to this problem, the molecular orbital method and the valence-bond method, only the latter which is the more closely related to the usual concepts of chemistry will be discussed. See Hückel, *Z. Physik*, **70**, 204; **72**, 310 (1931); **75**, 628 (1932); Pauling and Wheland, *J. Chem. Phys.*, **1**, 362 (1933); Wheland, *ibid.*, **2**, 474 (1934); Penney, *Proc. Roy. Soc. (London)*, **A146**, 223 (1934).

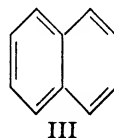
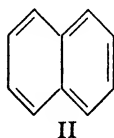
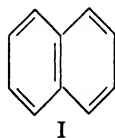
† It is convenient to call the valence-bond structures with the maximum number of double bonds *unexcited structures*, and those with a smaller number (the less important ones) *excited structures*.

C, *D*, and *E* make a small contribution, increasing the resonance energy to about 39,000 cal. per mole. This resonance energy makes the benzene ring about 39,000 cal. per mole more stable than a ring with three non-interacting double bonds, and gives it its peculiar aromatic properties.

As a result of resonance, each of the carbon-carbon bonds assumes properties similar to those of a double bond (except for the greater stability conferred by the resonance energy). Hence all the atoms in the molecule are restricted to one plane (as in ethylene, for example), and the bond angles are restricted to values near the tetrahedral angles $109^{\circ} 28'$ and $125^{\circ} 16'$. These conditions are satisfied by the six-membered ring of benzene. On the other hand, the angles of 90° and 135° in plane rings of cyclobutadiene and cyclooctatetraene, respectively, introduce so much strain as to counteract the stabilizing effect of resonance.²²

The quantum-mechanical treatment of benzene has been found to provide an explanation of many characteristic properties of the substance. On p. 1881 directed substitution is briefly discussed from this viewpoint. The effect of five-membered and six-membered saturated side rings in influencing the properties of benzene, discovered by Mills and Nixon,²³ has been shown²⁴ to depend on a change of a few per cent in the contributions of the two Kekulé structures to the resonating structure of the molecule.

Naphthalene, Anthracene, Phenanthrene. The three most important valence-bond structures for naphthalene are the following:



These contribute about equally to the resonating structure, the symmetrical structure I being somewhat more important than II and III. In addition smaller contributions are made by various less stable structures. This resonance stabilizes the molecule by 74,000 cal. per mole, giving naphthalene aromatic properties similar to those of benzene.²⁵ As in benzene, each carbon-carbon bond has properties approaching those for a double bond; the entire molecule is planar,²⁶ with bond angles near 120° .

²² Penney, *Proc. Roy. Soc. (London)*, **A146**, 223 (1934).

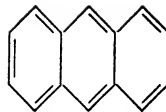
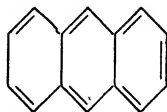
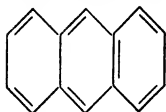
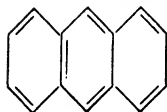
²³ Mills and Nixon, *J. Chem. Soc.*, 2510 (1930).

²⁴ Sutton and Pauling, *Trans. Faraday Soc.*, **31**, 939 (1935).

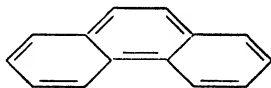
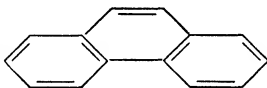
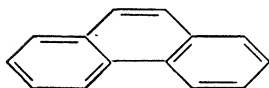
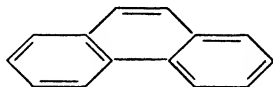
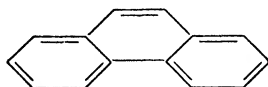
²⁵ Pauling and Wheland, *J. Chem. Phys.*, **1**, 362 (1933); Sherman, *ibid.*, **2**, 488 (1934).

²⁶ Robertson, *Proc. Roy. Soc. (London)*, **A142**, 674 (1933).

In anthracene four structures



make the largest contributions to the normal state of the molecule, smaller contributions being made by several hundred other structures. Five structures are important for phenanthrene:



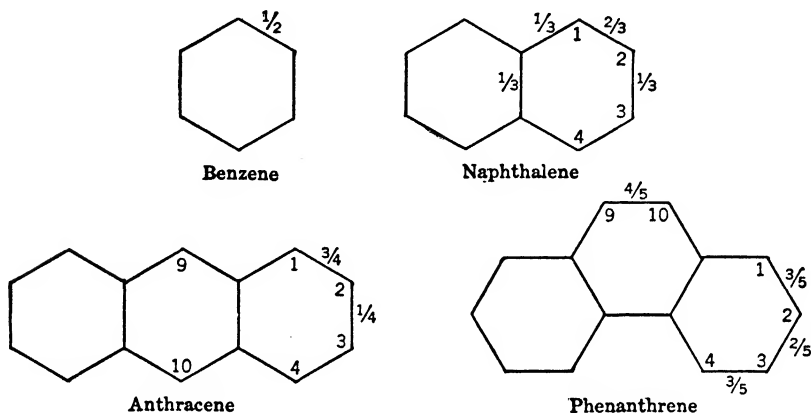
The resonance energies for these two molecules are 104,700 cal. per mole and 110,000 cal. per mole, respectively, both substances thus having aromatic properties, with phenanthrene about 5000 cal. per mole more stable than anthracene (Table I). The additional stability of phenanthrene is attributed to the fact that it resonates among five unexcited structures rather than four. It is also found that in larger polycyclic molecules greater stability accompanies more extensive branching, which increases the number of unexcited structures contributing to the resonance energy.

It must be emphasized that the larger resonance energy of naphthalene than of benzene does not require naphthalene to be more aromatic than benzene in its chemical reactions, inasmuch as the stabilizing action of the resonance energy is divided among five double bonds in the former and only three in the latter molecule. In general it is necessary to consider the resonance energy of the products of reaction also in discussing chemical properties of resonating molecules. Thus on hydrogenating benzene to 1,3-cyclohexadiene (with about 6000 cal. per mole resonance energy of conjugation of the two double bonds) there is a loss of resonance energy of about 33,400 cal. per mole, whereas the loss on hydrogenation of naphthalene in the 1,2 positions is only 29,200 (assuming 6000 cal. per mole energy of conjugation of double bond and benzene ring in 1,2-dihydronaphthalene). In consequence naphthalene may be expected to be more easily hydrogenated than benzene.* A very simple treatment of bond character in aromatic hydrocarbons which leads to conclusions in general agreement with the known chemical properties

* See Pauling and Sherman, *J. Chem. Phys.*, **1**, 679 (1933), for a more detailed treatment of hydrogenation.

of the substances can be made on the basis of the unexcited structures of the molecules. By examining the unexcited structures of an aromatic hydrocarbon to each bond may be assigned a fraction representing its double-bond character, this fraction being the ratio of the number of structures placing a double bond in this position to the total number of structures. This gives the following results*:

In benzene each bond has one-half double bond character, whereas in naphthalene the 1,2 bonds have two-thirds and the 2,3 bonds one-third



double-bond character. These numbers cannot be given a simple quantitative interpretation in terms of chemical reactivity; they do demand, however, that qualitative relations be satisfied. The 1,2 bonds in naphthalene must be much closer to ordinary double bonds in their properties than are the benzene bonds, which in turn are much more like double bonds than are the 2,3 bonds in naphthalene, the last, indeed, having practically no properties of a double bond. These statements are in agreement with general chemical experience. Various coupling reactions of naphthalene involving the 1,2 positions show the 1,2 bonds to have, to a pronounced extent, the properties of a double bond, whereas the 2,3 bonds show no such properties.²⁷

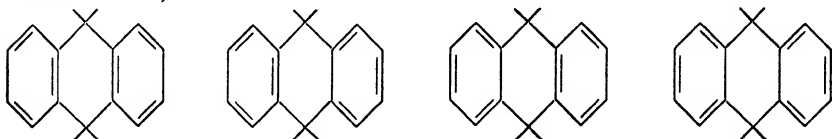
The 1,2 bonds in anthracene have a still more pronounced double-bond character,²⁸ which in turn is exceeded by that for the 9,10 bond in phenanthrene. This explains the fact that phenanthrene (despite its greater thermodynamic stability than anthracene, consequent to its greater resonance energy) is more reactive than anthracene.

* For a discussion of the dependence of interatomic distance on double-bond character see Pauling, Brockway, and Beach, *J. Am. Chem. Soc.*, **57**, 2705 (1935).

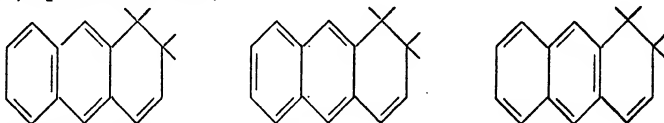
²⁷ Fieser and Lothrop, *ibid.*, **57**, 1459 (1935), and earlier references quoted by them.

²⁸ Fieser and Lothrop, *ibid.*, **58**, 749 (1936), and references quoted by them.

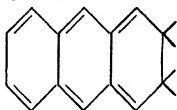
The activity of anthracene in the 9,10 positions cannot be discussed in this way. Instead may be considered the fraction of the number of unexcited structures for the product of an addition reaction at these positions in comparison with other positions. For the 9,10 positions there are four,



for the 1,2 positions three,



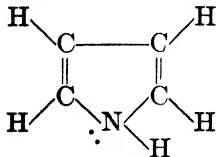
and for the 2,3 positions only one,



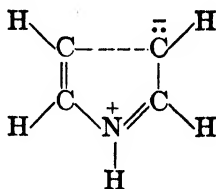
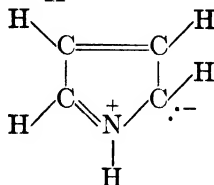
A large reactivity of anthracene toward addition reactions (and other reactions which involve these as intermediates) in the 9,10 positions is accordingly expected: smaller reactivity in the 1,2 positions; and negligible reactivity in the 2,3 positions. This is in agreement with experiment.

Heterocyclic Molecules. The resonance energies of pyridine and quinoline are about the same as for benzene and naphthalene, respectively, the same valence-bond structures contributing as for these molecules, and consequently the aromatic character being just as pronounced.

In pyrrole, furan, and thiophene, with resonance energies in the neighborhood of 20,000–30,000 cal. per mole, the structures other than



which make the largest contribution are of the types



the unshared electron pair resonating among all the atoms of the ring. Similar structures also contribute in indole, carbazole, and other heterocyclic compounds, giving rise to resonance energies of about the same magnitude as for aromatic hydrocarbons with the same number of rings.

Orientation of Substituents in Aromatic Molecules.* When a substituent is introduced into an aromatic molecule it may enter into certain of the available positions more readily than into others. This phenomenon has been exhaustively studied, and empirical rules have been found which describe the experimental results fairly satisfactorily (p.110). Thus in a monosubstituted benzene C_6H_5R the groups $R = CH_3, F, Cl, Br, I, OH, NH_2$, etc., are *ortho-para* directing, and $R = CO_2H, CHO, NO_2, N(CH_3)_3^+$, etc., are *meta* directing. Most *ortho-para* directing groups activate the molecule so that substitution takes place more readily than in benzene itself, and most *meta* directing groups have a deactivating effect. In naphthalene, substitution occurs at the α -position, in furan, thiophene, and pyrrole at the α -position, and in pyridine at the β -position, all of these molecules except pyridine being more active than benzene.

During the last fifteen years a qualitative theory has been developed† which accounts satisfactorily for the phenomenon in its major features, and recently a quantitative treatment based on the quantum mechanics has been carried out,²⁹ with a degree of success which provides strong support for the theory.

The theory is based on the consideration of the distribution of electric charge (the electron distribution) in the molecule in which substitution is taking place. In a benzene molecule the six carbon atoms are equivalent, and the charge distribution is accordingly such as not to make one carbon atom different from another. In the molecule C_6H_5R , with R attached to carbon atom 1, the electron distribution will in general be affected by the group R in such a way as to change the charges on the *ortho* (2 and 6) *meta* (3 and 5), and *para* carbon atoms. Moreover, the electron distribution may also be changed somewhat on the approach of the substituting group R' to one of the carbon atoms ("polarization" of the molecule by the group R'); in benzene the polarization of one carbon atom by the group would be the same as for another, but in a substituted benzene the polarization would in general vary from atom to atom, and so might cause a difference in behavior of

* The discussion in this section refers to substitution reactions involving the more common (cationoid) reagents.

† Many workers, including Fry, Stieglitz, Lapworth, Lewis, Lucas, Lowry, Robinson, and Ingold, have contributed to the theory. For an excellent review see Ingold, *Chem. Rev.*, **15**, 225 (1934).

²⁹ Wheland and Pauling, *J. Am. Chem. Soc.*, **57**, 2086 (1935).

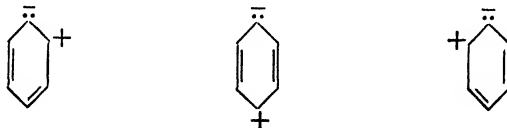
different positions. The fundamental postulate of the theory of orientation of substituents is the following: *In an aromatic molecule undergoing substitution by the group R', the rate of substitution of R' for hydrogen on the *i*th carbon atom increases with increase in the negative charge on the *i*th carbon atom when the group R' approaches it.*

Substitution is thus assumed to take place preferentially on that carbon atom on which the negative charge is the largest. There are two principal ways in which the charge distribution can be affected by the group R, for each of which it has been assumed, and has been verified by quantum-mechanical calculations,* that the *ortho* and *para* carbon atoms are about equally affected, the *meta* carbon atoms being affected to a much smaller extent.

The first effect of the group R, called the *inductive effect*, results whenever the electron affinity of the group is larger than or smaller than that of hydrogen. In the former case electrons are attracted to the group and to the attached carbon atom 1; calculation shows that they are removed mainly from the *ortho* and *para* carbon atoms.† Consequently the rate of substitution at the *ortho* and *para* positions will be greatly decreased and that at the *meta* positions somewhat decreased; the group R will be *meta* directing, with deactivation. An example of such a group is $\text{N}^+(\text{CH}_3)_3$, in trimethylphenylammonium ion; the nitrogen atom has a larger electron affinity than hydrogen, and its attraction for electrons is further intensified in this case by its positive charge. The same effect is seen in pyridine; the nitrogen atom attracts electrons mainly from the α and γ carbon atoms, and consequently pyridine substitutes in the β positions, and is less active than benzene. Toluene shows the opposite effect. Electric moment measurements show that the methyl group loses electrons to the ring; these go mainly to the *ortho* and *para* carbon atoms, which are thus activated, toluene

* Wheland and Pauling, *loc. cit.* This was first shown, for the inductive effect alone, by Hückel, *Z. Physik*, **72**, 310 (1931).

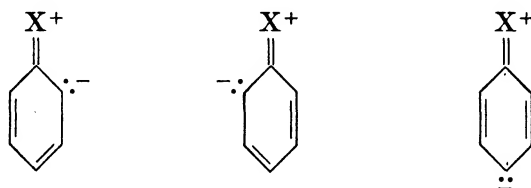
† This result can be seen from the following qualitative argument. An excess negative charge attracted to carbon atom 1 is accounted for by resonance to ionic structures in which this atom has an unshared pair. There are only three unexcited ionic structures of this type,




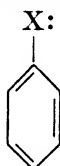
and they correspond to removing electrons equally from the two *ortho* atoms and the *para* atom. The *meta* atoms remain unaffected so long as the excited ionic structures are not considered; their effect would be small.

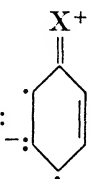
substituting in these positions, and substitution occurring with greater ease than in benzene.

It might be expected that F, Cl, Br, I, OH, and NH_2 would be *meta* directing, inasmuch as these groups all have larger electron affinities than hydrogen. Actually they are all *ortho-para* directing, the inductive effect for them being overcome by another effect, called the *resonance effect* (or sometimes the *tautomeric* or *electromeric effect*, p. 1617). For the molecules considered in the preceding paragraph a consideration of resonance was not needed, except to the same extent as in benzene itself. If the group R possesses an unshared pair of electrons, however, other structures make an appreciable contribution to the normal molecule.* Thus the structures



in which the unshared pair resonates to the *ortho* and *para* positions are

almost as stable as  and , each possessing three double

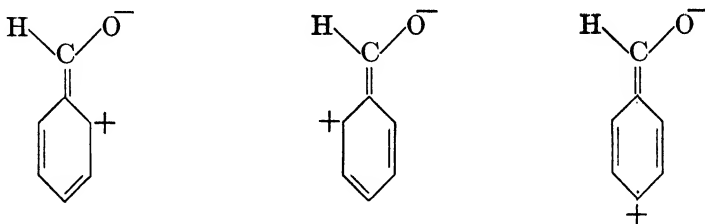
bonds. (Excited structures such as: , with two electrons not

involved in a bond between adjacent atoms, are much less stable and need not be considered.) These three additional structures increase the electron density on the *ortho* and *para* atoms, and so make the groups *ortho-para* directing, the resonance effect being more significant than the inductive effect.

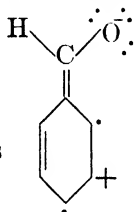
In benzaldehyde and many similar molecules, on the other hand, resonance directs toward the *meta* positions, this effect of resonance resulting whenever the substituted group R contains an electro-

* The contribution of these structures to the resonance energy amounts to about 6000 cal. per mole (Table I, phenol and aniline).

negative atom and a double bond conjugated with the benzene ring * ($R=CO_2H$, CHO , NO_2 , $COCH_3$, CN , etc.). The structures leading to this effect are of the type



which decrease the electron density in the *ortho* and *para* positions, thus permitting reaction at the *meta* positions. (The contribution of excited



structures such as

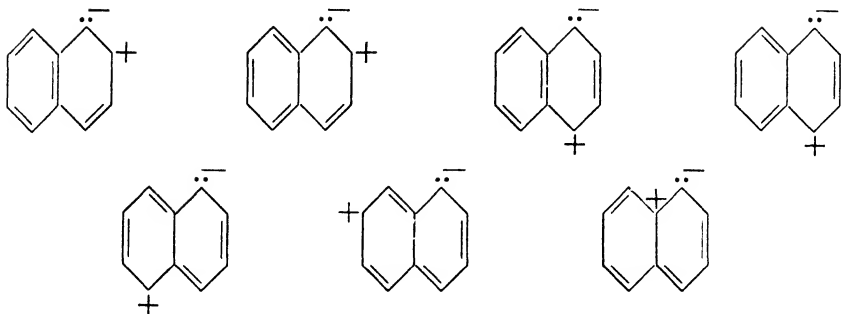
is small because of their instability; it

suffices to produce some deactivation, however.)

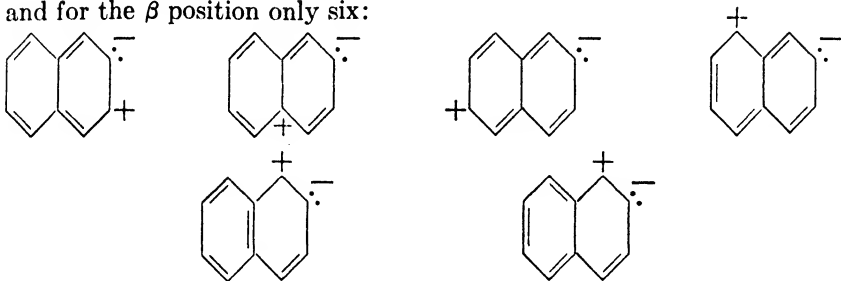
So far only the permanent charge distribution, as influenced by the inductive and resonance effects, has been discussed. The discussion for a monosubstituted benzene can be summarized as follows. When resonance does not occur, substitution is usually determined by the inductive effect, an electron-attracting group ($N^+(CH_3)_3$) being *meta* directing and an electron-repelling group (CH_3) *ortho-para* directing. The resonance effect, which when present is usually more powerful than the inductive effect, is *meta* directing when the group contains a double bond conjugated with the benzene ring, and *ortho-para* directing when the group contains an unshared electron pair on the atom adjacent to the benzene ring.

In a few cases (naphthalene, for example) it is necessary to consider also the polarization of the molecule by the attacking group; as yet no general qualitative rules have been formulated regarding this effect, though some quantitative calculations have been carried out. The effect can be treated qualitatively by the consideration of the number of unexcited ionic structures placing an unshared pair on the carbon atom being attacked. For the α position of naphthalene there are seven:

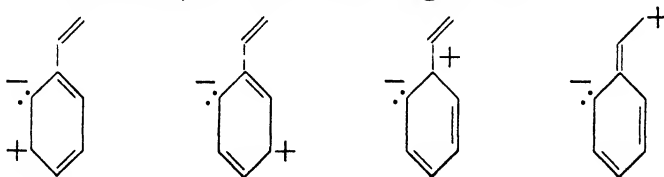
* The resonance energy of this conjugation amounts to 5000 — 10,000 cal. per mole (Table I).



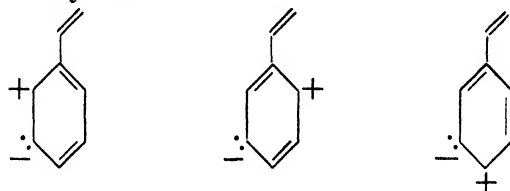
and for the β position only six:



hence the polarization by the attacking group will be greater for the α position, and substitution will take place there. The same argument can be applied to phenylethylene, which is *ortho-para* directing. Its zero electric moment shows that the vinyl group has no pronounced difference in electron affinity from hydrogen, and so will produce no inductive effect, and calculation shows that there is no resonance effect (which depends on the presence of an electronegative atom as well as of the double bond). However, polarization is greater in the *ortho* and *para* positions than in the *meta* positions, the former having four unexcited ionic structures, such as the following for *ortho*:



and the latter only three:



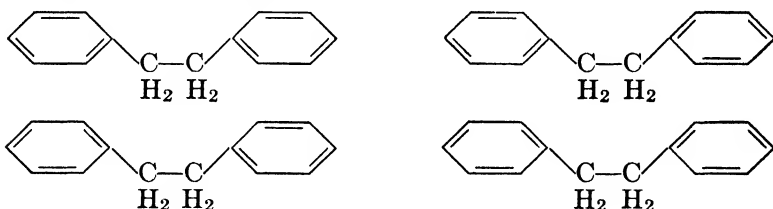
The Hydrocarbon Free Radicals (p. 489). The modern theory of the stability of the aromatic free radicals is based on resonance.* Increase in the degree of dissociation of a substituted ethane, R_3C-CR_3 , might result either from a decrease in stability of the undissociated molecule or an increase in stability of the products of dissociation, the free radicals R_3C . For the hexaalkylethanes, which

do not dissociate easily, the electronic structure $\begin{array}{c} R & & R \\ & \diagdown & / \\ R-C & - & C-R \\ & / & \diagdown \\ R & & R \end{array}$ is written,

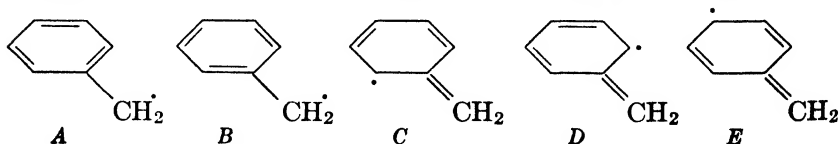
and for the corresponding free radical the structure $\begin{array}{c} R \\ | \\ R-C\cdot \\ | \\ R \end{array}$, the odd

electron (free valence) being located on the methyl carbon atom. The introduction of an aryl group as a substituent R , however, provides additional structures for the radical; it is principally the energy of resonance among these which stabilizes the free radical and increases the degree of dissociation of the substituted ethane.

For simplicity, the molecule $C_6H_5CH_2-CH_2C_6H_5$, *sym*-diphenylethane, may be considered, and the discussion of resonance may be restricted to the structures with the greatest stability (those with the maximum number of double bonds). For the undissociated molecule there is resonance among the four Kekulé structures,



whereas each of the free radicals can resonate among the five structures:



If the radical were restricted to resonance between the Kekulé structures

* The idea was developed empirically by Burton and Ingold, *Proc. Leeds Phil. Lit. Soc. Sci. Sect.*, **1**, 421 (1929); Ingold, "*Ann. Repts. Chem. Soc. (London)*," **25**, 154 (1928), and was put on a quantitative basis by the quantum-mechanical calculations of Pauling and Wheland, *J. Chem. Phys.*, **1**, 362 (1933), and Hückel, *Z. Physik*, **83**, 632 (1933).

A and *B*, with the free valence on the methyl carbon, resonance would stabilize the radicals to just the same extent as the undissociated molecule, which would then have only the same tendency to dissociate as a hexaalkylethane. But actually the five structures *A*, *B*, *C*, *D*, and *E* (each with three double bonds) contribute about equally to the structure of the radical, which thus resonates among five structures (instead of two), and is accordingly stabilized by the additional resonance energy, which is found on calculation to be about 15,000 cal. per mole.

The effect of two phenylmethyl radicals in decreasing the energy of dissociation by about 30,000 cal. per mole is not large enough to cause dissociation to an appreciable extent, inasmuch as the energy required to break the carbon-carbon bond in ethane is of the order of magnitude of 85,000 cal. per mole. In triphenylmethyl, however, the odd electron can resonate among nine positions (the *ortho* and *para* positions of the three phenyl groups) in addition to that on the methyl carbon atom; this leads to an additional resonance energy of about 38,000 cal. per mole, so that two such radicals stabilize the system by an amount (76,000 cal. per mole) sufficient to decrease the dissociation energy to only a few thousand calories per mole, resulting in extensive dissociation. In tribiphenylmethyl, in which the odd electron resonates among nineteen positions, the dissociating effect is still larger, the additional resonance energy being about 44,000 cal. per mole.

The quantum-mechanical discussion has been carried out by two distinct methods, the results of which are in essential concordance.³⁰ The detailed agreement with experiment in regard to such fine points as the greater dissociating action for α - than for β -naphthyl and for two phenyls than for fluoryl leaves little doubt that resonance of the odd electron (free valence) among several positions in the radical is the principal influence stabilizing the free radicals. It is also possible that other factors, such as the steric effects of the large groups, have a considerable influence in increasing the degree of dissociation.*

The positive and negative free radical ions have about the same possibilities of resonance as the free radicals themselves, the positive or negative charge (the latter being an unshared pair of electrons) resonating among the same positions as the odd electron; so that for all free radicals about the same values of the ionization potential and the electron affinity may be expected.³¹ This conclusion is in agreement

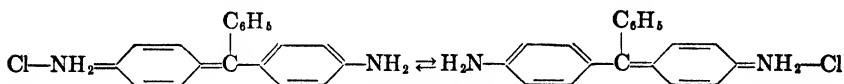
³⁰ Wheland, *J. Chem. Phys.*, **2**, 474 (1934).

* For a discussion of these points see Wheland, *ibid.*, **2**, 474 (1934), and Bent and Ebers, *J. Am. Chem. Soc.*, **57**, 1242 (1935).

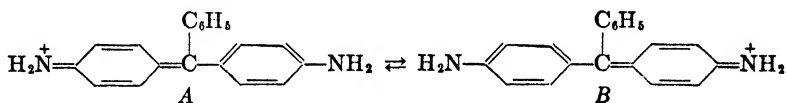
³¹ Wheland, *loc. cit.*; Pauling and Wheland, *J. Chem. Phys.*, **3**, 315 (1935); Hylleraas, *ibid.*, **3**, 313 (1935).

with the experimental results obtained by Bent,³² who has found values of about 60,000 cal. per mole for the electron affinity of several different free radicals.

Chromophores and Auxochromes.* It has been gradually recognized that the intense color of the triphenylmethane dyes and of other dyes whose constitution is well understood is closely related to resonance. Baeyer³³ suggested that in *p,p*-diaminotriphenylcarbinol hydrochloride (Döbner's violet), for example, the color is due to the oscillation of a chlorine atom from one end of the molecule to the other.



With the recognition of the ionic character of the bond to chlorine, this suggestion was revised by Adams and Rosenstein,³⁴ who correlated the color with an oscillation of an electron.



From the modern point of view intense color is the result of a transition of the molecule from its normal electronic state to an excited electronic state, with absorption of light, the transition having a very high probability if the electric-moment matrix element associated with it is large. Now the two valence-bond structures *A* and *B* represented above are equivalent; hence neither one represents the normal state of the molecule, which instead is represented by a combination of the two. There is also another combination of the same structures which represents an excited state of the molecule. Now it can be shown that the electric-moment matrix element associated with transition between these two states is very large, by the following argument. The negative ion may be considered to be near the center of the molecule.† Then structure *A* corresponds to a very large dipole moment (p. 1747) in one direction and *B* to the same moment in the other direction. The actual molecule in its normal state and the excited state under consideration will have zero moment, however, because with equal resonance between *A* and *B* their moments neutralize each other. It can be shown by quantum-mechanical methods that under these circumstances the

³² Bent, *J. Am. Chem. Soc.*, **52**, 1498 (1930); **53**, 1786 (1931).

* Bury, *ibid.*, **57**, 2115 (1935). We have extended Bury's discussion with the argument given in the second paragraph.

³³ Baeyer, *Ann.*, **354**, 152 (1907).

³⁴ Adams and Rosenstein, *J. Am. Chem. Soc.*, **36**, 1472 (1914).

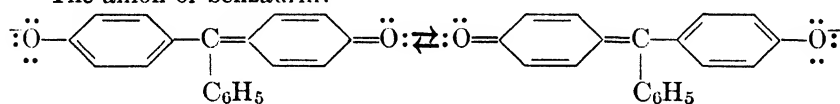
† This assumption is not necessary, but simplifies the argument.

electric-moment matrix element associated with the transition between the normal and the excited resonating state has the same magnitude as the moment associated with structures *A* and *B*, and is hence in this case very large; consequently the substance is very deeply colored, with the absorption of light corresponding to this electronic transition. The results of this argument can be summarized by saying that deep color results from resonance between two equivalent or nearly equivalent structures with which a large dipole moment is associated (the actual electronic transition being between resonating structures formed from these.)

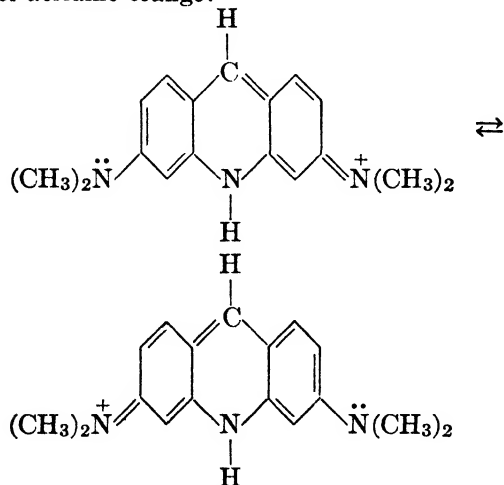
The division of groups into chromophores and auxochromes is rather arbitrary. Willstätter and Piccard³⁵ pointed out that some substances containing a quinoid chromophoric group, such as fuchsonimine, $\text{HN}=\text{C}_6\text{H}_4=\text{C}=(\text{C}_6\text{H}_5)_2$, are colorless or feebly colored, becoming strongly colored on introduction of another group (such as NH_2) called an auxochrome. Bury (*loc. cit.*) pointed out that in these cases the function of the auxochrome is to introduce the possibility of resonance.

Nitrogen and oxygen atoms are important in dyes in order to introduce the large electric moments. The structures which by resonance give the normal and significant excited states for some dyes are listed below; for others the reader is referred to Bury's article.

The anion of benzaurine:

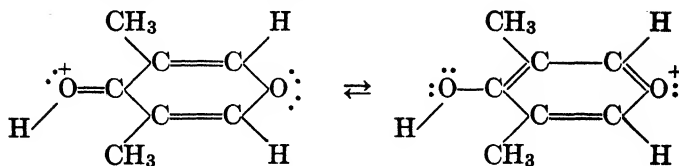


The cation of acridine orange:



³⁵ Willstätter and Piccard, *Ber.*, **41**, 1458 (1908).

The dimethylpyronium cation:



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MULLIKEN, *J. Chem. Phys.*, **1**, 492 (1933); **3**, 375, 506, 514, 517, 573, 586 (1935).

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